PHYTOTOXINS PRODUCED BY PATHOVARS OF *PSEUDOMONAS SYRINGAE*

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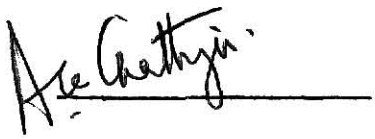

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THIS BOOK CONTAINS NUMEROUS PAGES WITH DIAGRAMS THAT ARE CROOKED COMPARED TO THE REST OF THE INFORMATION ON THE PAGE.

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Part I

Perspective

Man is dependent upon living plants for his survival. The destruction of plants used for food, fiber and recreation threatens man's ability to enjoy a healthy life. The causes of this destruction are chiefly insect and weed pests and disease. Fungi, bacteria and viruses are most important in terms of distribution, diversity and total damage to plants, both in the field and in storage (64). Many fungi and bacteria are capable of producing toxic compounds which contribute to disease symptoms. During the past decade, the volume of literature concerning phytotoxins has increased dramatically. Previous to this, only sporadic reports were published which dealt mainly with biologic activity of proposed phytotoxic compounds (64). Advances in technology and integration of knowledge from organic chemistry, biochemistry, plant physiology and plant pathology have added to the available literature on the cmistry, mode of action and role of phytotoxins. Still, the amount of research accomplished is minute when compared to the body of knowledge on antibiotics and other antimicrobial compounds known to be of economic and industrial significance.

To illustrate the need for increased research on phytotoxins, one need only consider the southern corn leaf blight epidemic of 1970-1971. This episode had a greater one-year impact on the economy of the U.S. than any other plant disease or pest in history. Toxin produced by *Helminthosporium maydis* (syn. *Bipolaris maydis*) was a major factor in the destructive

process (55). Phytotoxins may also be useful in the study of the physiological and biochemical activities of plants. They have been used in selecting seeds (64), tissues and regenerated plant parts for disease resistance or tolerance (6).

Several mechanisms are currently recognized by which pathogenic microorganisms may cause disease (1, 55): 1) production and release of substances that interfere with metabolism, alter the normal constitution of the protoplasm or alter the growth, development and reproduction of the host plant, 2) production and release of enzymes that degrade plant tissues and cell wall components, and 3) cause dysfunction of the normal movement of water, nutrients and metabolites. Generally, bacterial phytotoxins produce disease symptoms through the interference of metabolism or they alter the constitution of the protoplasm of the host plant cells.

The term toxin may be interchanged with antibiotic when referring to a chemical compound which retards or inhibits the growth of an organism. For purposes of this discussion, a toxin is considered as a microbial product other than an enzyme, which causes disease development (55). A phytotoxin, then, is a toxin produced by a phytopathogenic organism which can be associated with disease development in the host plant. Bacterial phytotoxins are compounds of diverse size and chemistry (see below); all described so far are not host specific and have been implicated in plant disease.

Whether a phytotoxin is responsible for pathogenicity or contributes to virulence has not been determined in all cases. Most textbooks refer to the ability of an organism to induce disease as pathogenicity. virulence describes quantitatively the relative disease-inducing ability of an organism. This approach will be followed herein.

There are several indications of toxin involvement in plant disease: chlorosis, wilting, brightly colored lesions and the pathogen being well-removed from the site of the symptom(s). To implicate toxin involvement in disease most, preferably all, of the following criteria should be met (70): 1) Toxin-induced symptoms should resemble those in the naturally infected plant. 2) One should be able to isolate toxin from diseased plants. 3) The quantities of toxin present *in vivo* must be large enough to account for the symptoms. 4) Non-toxigenic strains should not produce toxin related symptoms but should be able to colonize the host. It may be possible to find genetic variants of the host that are insensitive to the toxin (55, 64).

This report will focus on the phytotoxins produced by various pathovars of *Pseudomonas syringae*. The presence of most antibiotics or toxins from various organisms cannot be demonstrated in the natural environment of the producing organism. It has been demonstrated that some phytotoxins are produced by pseudomonads in infected plants. This may confer a selective advantage, enabling the bacterium (in conjunction with other unknown factors) to colonize specific host plants i. e. ecological niches not otherwise accessible (27, 29). The ability to demonstrate the presence of these compounds in plants and in culture vessels proves pseudomonads to be good candidates for the further study on the mechanisms and physiology of toxin production. Toxin producing pseudomonads are also useful as they do not undergo cellular differentiation which occurs in bacilli, fungi and actinomycetes (29).

Pseudomonas syringae belongs to the most studied group of pseudomonads, the fluorescent pseudomonads. Included in the group are: *P. aeruginosa*, the type species, *P. fluorescens*, *P. putida*, *P. chlororaphis*,

P. aureofaciens and P. cichorii, the only other phytopathogenic fluorescent pseudomonads. All of the fluorescent pseudomonads fall into one five RNA homology groups which are defined by rRNA-DNA competition experiments. The guanine-plus-cytosine content of their DNA ranges from 59-67 mol %. Together with other pseudomonads, they are Gram-negative, strictly aerobic, polarly flagellated rods. Other than these characters, the group only has one common character, the ability to produce water soluble fluorescent pigments (29).

The pathovars of Pseudomonas syringae are pathogenic on a wide range of plant species, both dicots and monocots. The toxins produced, which have been characterized, are equally heterogenous (Table 1). This report will consider those toxins that have been characterized or partially characterized, their mode(s) of action, genetics, metabolism and their similarities to antibiotics and toxins produced by nonphytopathogenic microorganisms.

Characteristics of Selected Phytotoxins

Tabtoxin

Tabtoxin (Figure 1) is a dipeptide of tabtoxinine-β-lactam linked to either threonine or serine. It produces the characteristic chlorosis of wildfire disease of tobacco caused by P. syringae pv. tabaci (63). Tabtoxin-producing bacteria may also originate from various hosts such as soy bean and bean. Strains of P. syringae pv. coronafaciens originating from oats, maize and timothy (51, 67) also produce tabtoxin and production has been reported from P. syringae pv. garcae, a pathogen of coffee (34).

The labile β-lactam group on one of the amino acid residues causes tabtoxin to be chemically unstable. This has made isolation and characterization difficult. Isotabtoxin, the biologically inactive isomer (Figure 2), is formed in aqueous solutions at pH levels above 7. Isomerization is minimal at pH 3-3 and at low temperature (i.e. $4^{o}C$) (34).

In the laboratory, tabtoxin is generally produced in liquid culture at $25^{o}C$. Toxin is isolated from the culture supernatant by cation-exchange resin, eluted with 5% ammonia then purified by ion-exchange chromatography (63).

Strong acid hydrolysis (6N HCl, $100^{o}C$) of tabtoxin yields the amino acids threonine and tabtoxinine, a previously uncharacterized amino acid (67). Mass spectrometry, oxidative degradation studies and nuclear magnetic resonance (nmr) data were used to reveal the structure of the toxin. The inactive isomerization product from tabtoxin has a δ-lactam structure arising from the intramolecular translactamization reaction between the dipeptide α-amino and β-lactam carbonly groupings (28). Along with tabtoxin, P. syringae pv. tabaci and other producing strains, the serine

analog is produced, (2-serine)tabtoxin (Figure 3). This analog is composed of tabtoxinine and serine and possess the same biological activity as tabtoxin. Since they are formed simultaneously in culture and have equal biological activities (67), "tabtoxins" collectively describes these analogs.

Tabtoxins cause a light-dependent chlorosis in plants (11), accumulation of ammonia in infected tissue, inhibition of the alga Chlorella vulgaris (57), convulsions in mammals (59) and inhibition of Escherichia coli strain K-12 (15). The mode of action, however is still unclear. Initially, tabtoxins were thought to effect methionine metabolism due to their resemblance to methionine sulfoximine (MSO), a potent methionine antagonist (5). Closer inspection showed that though MSO could compete with methionine at the plasmalemma, the real site of action is glutamine synthetase (E.C. 6.3.1.2., abbr. GS). GS is integral in nitrogen assimilation and regulation in living cells. It catalyzes the reaction whereby ammonia is added to L-gutamate to produce L-glutamine. Energy for the reaction is supplied by the hydrolysis of ATP to ADP and orthophosphate. Crude tabtoxin preparations supported the MSO hypothesis (31). However, highly purified GS, tabtoxin and a more sensitive assay did not show inhibition of GS (69). This problem was resolved when it was noted that the free β-lactam form of tabtoxinine, tabtoxinine-β-lactam, is found in culture filtrates of P. syringae pv. tabaci. It has equal capacity to induce chlorosis and possesses all the structural features of tabtoxins that are required for biologic activity (12). From this, it was concluded that tabtoxinine-β-lactam may not be the primary bacterial product, rather it is produced by peptidase cleavage by bacterial or plant enzymes (69). The β-lactam form may well be the active toxin in vivo as demonstrated by the case of

phaseolotoxin being cleaved to (N^{δ}-phosphosulphamyl)ornithine (34) (to be discussed).

Turner (68) in an attempt to identify the mechanism by which chlorosis is induced and if, indeed, GS is inhibited, reported on the effects of tabtoxin on Nicotiana tabacum leaves. When toxin was infiltrated into intact leaves, tissue necrosis was evident after 30 hours and continued until 100% of the infiltrated tissue was necrotic, about 60 hours later. GS activity decreased by 16.5% one hour after toxin infiltration and 95% loss of activity occurred after four hours. Ammonia started to accumulate three to four hours after toxin treatment, when GS activity had decrease by 5-10% of the controls. Ammonia accumulation increased during the light cycle. Free amino acids accumulated in small but reproducible amounts after the first few hours, then rose suddenly to 30% higher than the controls after 36 hours.

After 72 hours in leaves injected with tabtoxin, ammonia accumulation was nearly zero. Tissues harvested at this time showed an inverse relationship between GS activity and ammonia levels. As toxin concentrations increased, GS activity decreased and higher levels of ammonia accumulated. The dose of toxin required for chlorosis also initiated the accumulation of ammonia.

Tabtoxin thus may play a role in the colonization of N. tabacum by P. syringae pv. tabaci. High levels of ammonia are made available for bacterial utilization through the inhibition of GS activity. The ability of the host to respond to the pathogen may be impared as a result of a weakened ability for de novo protein synthesis due to lowered glutamine levels

Syringomycin, Syringotoxin

Syringomycin (SR) (Figure 4) is produced by P. syringae pv. syringae. The extent of SR production varies from strain to strain but is limited to P. syringae pv. syringae that are pathogenic on stone fruit, pear and grass hosts (21). SR has a wide spectrum of antibiotic activity. Six bacterial species, four fungal species, a unicellular alga and a Daphnia species are inhibited by low levels of SR (13, 58). A sensitive organism, Geotrichum candidum, is used as a bioassay for SR. Though the amount of SR required to cause inhibition of G. candidum is five times that required to cause lesions in maize plants (58). When toxin, which has been purified from liquid culture by ion-exchange chromatography and partition chromatography, is applied to host plant tissue, typical P. syringae pv. syringae symptoms appear: necrosis in peach shoots, water-soaking and necrosis in maize (58). Reisolation of an antibiotic, indistinguishable from SR by paper chromatography, from diseased plants indicated structural similarity of the toxins produced in vivo and in vivo (58).

Analysis of the purified toxin indicates positive charges on the molecule at pH 4. Strong acid hydrolysis yields four amino acids: serine, phenylalanine, arginine and an as of yet unidentified basic amino acid, probably lysine with a substitution on the R group, in a molar ratio of 2:1:1:2. It is most likely a hexapeptide (34). Upon alkali treatment, biological activity is lost (58). No change of amino acid composition can be noted; it is therefore suggested that there may be ionizing groups or bonds present that were undetected in earlier studies. Microanalytical data suggest the presence of a highly oxygenated substituent which contains little nitrogen. Mitchell (34) suggests the

butyl amino group of lysine is substituted by a glucosyl residue, since the caculated molecular weight of the peptide, without substitutions and including lysine, is considerably lower than that obtained by micro-analysis, and the proportion of nitrogen present is much lower than that of carbon and oxygen.

Members of P. syringae pv. sryingae that are pathogenic on citrus produce, instead of SR, syringotoxin (ST) (Figure 4). It has never been reported that strains or isolates producing SR in culture produce ST or vice versa. Biological activity of ST is comparable of SR, that is, water-soaking, chlorosis and necrosis of maize leaves and inhibition of eukaryotic cells. G. candidum is also a means of ST assay (19).

Alkali treatment of ST also causes loss of biological activity, A different complement of amino acids are liberated upon strong acid hydrolysis. Equal molar proportions of threonine, serine, glycine and ornithine are obtained. Another component, as yet unidentified is also present (20, 34).

There is evidence that the mode of action of SR and ST have a few similarities, though they are by no means identical. Surico and Devay (65) noted changes in the oxidative metabolism of mitochondria from etioliated shoots of two cultivars of maize, one resistant and one susceptible to holcus spot disease. The effects of the two toxins were comparable on both types of mitochondria.

The results of several experiments showed both toxins to have the same general effect on isolated mitochondria: 1) respiration was stimulated with succinate, NADH and malate/glutamate as respiratory substrates; 2) respiratory control and ADP:O ratios were reduced. At very high toxin

concentrations respiratory control ratio was 1, ADP:O ratio was 0. At these concentrations, the further addition of ADP had no effect on respiration rate.

The concentration of SR and ST influenced the speed and magnitude of respiration rate increase. When the toxin concentration was increased or was preincubated with mitochondria before addition of substrate, there was almost no lag phase before an increase in respiration took place. SR without preincubation at lower concentrations generally had a longer lag phase. AT also produced higher rates of respiration than SR. This may indicate differences in the rate of entry of SR and ST, or in accessibilities to their sites of action, their specificity or their mechanisms.

Resistant mitochondria showed stimulation of ATPase activity, inhibition of ATP formation, swelling of mitochondria and a loss of membrane potential in the presence of SR and ST. These resistant mitochondria, when treated with ST and in the presence of 0.2 M KCl, showed much more swelling than when treated with ST at the same or lower concentrations. At concentrations that affected oxygen uptake, the swelling was minimal for both. This swelling, then, is probably not a result on respiration of the toxins; rather, it may indicate differences of mechanisms. Potassium ions present may also affect the degree of swelling.

The effects on mitochondria, ATPase activity and ATP formation by SR and ST parallel those of classical uncoupling agents such as dinitrophenol (DNP). Since they are surface active (2), SR and ST cause an increase of membrane permeability thus disrupting the ion gradient across the membrane. The concentrations of SR and ST required to cause

loss of respiratory control did not have a great effect of membrane potential. In the presence of high concentrations of NADH and ADP, ATP was still synthesized though complete uncoupling of oxidative phosphorylation occurred at high toxin concentrations, where the action of SR and ST was irreversible. This may be the reason why at low levels of SR and ST, which caused loss of respiratory control, membrane potential was not greatly affected.

In pathogenicity tests, the susceptible maize cultivar supported higher bacterial populations than the resistant cultivar (65). Numbers of lesions and the time required for lesion development were greater in the susceptible cultivar. There were no major differences in the sizes of the lesions. In phytotoxicity tests, both purified SR and ST showed more severe effects on the resistant line, dramatic holcus spot disease symptoms appeared: water-soaked lesions which later turn light-colored and papery. In this case, resistance to holcus spot disease was due to the inability of the pathogen to survive in the host, not a resistance mechanism or gene in the plant. SR and ST appear to be involved in virulence; the ability to colonize the host is determined by other factors.

Phaseolotoxin

P. syringae pv. phaseolicola, the bean halo blight organism, produces a chlorosis-inducing toxin. The compound is purified from culture supernatants by adsorbtion onto charcoal and recovered by extraction with methanol:chloroform:ammonia. The recovered liquid is subjected to ion-exchange chromatography on QAE Sephadex and gradient elution with NH_4CO_3. This is followed by partition chromatography on Sephadex LH 20 using methanol-aqueous ammonia. The structure has been determined to be

(N^{δ}-phosphosulphamyl)ornithylalanylhomoarginine. The trivial name has been given as phaseolotoxin (Figure 5) (PSLT) (32). On occasion, however, nontoxigenic strains have been isolated (33, 60). No other pathovars of P. syringae have been reported to produce PSLT (34).

Also found to some extent (5-10%) in culture filtrates of P. syringae pv. phaseolicola is (2-serine)phaseolotoxin. This analog also causes chlorosis in bean leaves and has been purified to homogeneity, Serine replaces alanine in the tripeptide (Figure 6). (2-serine)phaseolotoxin behaves similar to PSLT upon acid hydrolysis and peptidase treatment (33).

The active component causing chlorosis in leaf tissue is (N^{δ}-phosphosulphamyl)ornithine (Figure 7). Phaseolotoxin and (2-serine)phaseolotoxin are present at low levels in diseased plant tissues. As (N^{δ}-phosphosulphamyl)ornithine is undetectable in culture filtrates, the primary in vivo toxin appears to be the product of secondary enzymatic hydrolysis of the microbial product, PSLT, by enzymes of plant or bacterial origin (36).

Unique to this molecule and responsible for its biologic activity is the N-phosphosulphamyl group (33). The tripeptide backbone is not associated with activity, rather, it may function in transport of the toxin across plant cell or organelle membranes via a peptide permease (43).

The diagnostic chlorotic halo symptom of halo blight in bean leaves is a result of the ability of the toxin to competitively bind the enzyme L-ornithine carbamoyltransferase (E.C. 2.1.3., abbr. OCT) which catalyzes the conversion of ornithine to citrulline. The sulphamyl phosphate group mimics carbamyl phosphate which is enzymatically added to ornithine to produce citrulline and inorganic phosphate. This reaction is common

to arginine biosynthesis and the urea cycle. Ornithine accumulates in infected or toxin-treated bean leaf tissues due to the inhibition of this reaction (38, 53).

Phaseolotoxin is inhibitory against a range of microorganisms, including Euglena gracilis (52), Salmonella typhimurium and Escherichia coli (60). There is a complete correlation between inhibition of E. coli and chlorosis induction in bean plants. The inhibition of OCT causes a phenotypic requirement for arginine in minimal glucose salts medium which can be reversed by the addition of arginine or citrulline, but not by ornithine, to the E. coli culture medium. A linear relation exists between the amount of inhibition of E. coli and the amount of PSLT. By this assay as little as 10-12 pg of purified toxin could be detected.

PSLT bears structural resemblance to an antimetabolite isolated from fermentation broths of an unclassified Streptomyces species (Figure 8) (49). The antibiotic, which shows activity against Serratia sp. and Bacillus subtilis, has the structure L-(N^5-phosphono)methionine-S-sulfoximinyl-L-alanyl-L-alanine. It is produced in a defined minimal medium and the effects can be reversed by the addition of a specific L-amino acid, L-glutamine (49). Its tripeptide nature, phosphate substituted amino acid, production in minimal medium and reversal by a specific amino acid parallel the characteristics of PSLT. PSLT is hydrolyzed by carboxypeptidase to phosphosulphamyl ornithine; likewise, this streptomycete antibiotic is susceptible to enzymatic hydrolysis by carboxypeptidase and leucine amino peptidase.

Tagetitoxin

Apical chlorosis in marigolds and zinnias infected with P. syringae

pv. tagetis is the result of a toxin. Mitchell and Durbin (37) isolated, purified and chemically characterized this toxin which they gave the trivial name tagetitoxin. Its structure has not yet been reported. Purified toxin, when applied via a pinprick wound to zinnia or marigold seedlings, produces apical chlorosis typical of P. syringae pv. tagetis infection.

Tagetitoxin can be produced in liquid culture in a defined medium. Extraction of the toxin is rather difficult as it is hydrophylic, non-extractable into organic solvents and is acid labile (34). These problems hindered early work on the purification and characterization of tagetitoxin. Extraction and purification steps include partitioning the concentrated culture supernatant into various proportions of methanol: chloroform:water. After careful manipulation of the extraction system, the dry weight of the toxin fraction, without too much loss of activity can be reduced to an amount small enough for gel filtration on BioGel P2. This affords a 5-fold concentration of activity. The next step in purification is ion-exchange chromatography on DEAE Sephadex and gradient elution with NH_4HCO_3, The final step is partition chromatography on Sephadex LH 20 using methanol-aqueous ammonia (37).

Purified tagetitoxin, when subjected to thin-layer chromatography on cellulose sheets, reacts purple with ninhydrin indicating the presence of an amino group. Tagetitoxin also reacts with molybdate indicating a phosphate group. At pH 2, tagetitoxin is electrically neutral, but migrates toward the cathode during electrophoresis at pH 7. Biological activity is lost upon dilute acid treatment at $20^{o}C$. The molecular formula is believed to be $C_{11}H_{18}O_{13}SNP$. This formula was arrived at by phos-

phorous nmr, ^{13}C nmr, proton nmr and ^{35}S incorporation studies. Field desorption-mass spectroscopy provides a tentative molecular weight of 435 (37).

Coronatine

The chlorosis response of various grasses infected with P. syringae pv. atropurpurea is caused by the toxin coronatine (Figure 9). Coronatine causes hypertrophy of isolated potato cells at concentrations of 1×10^{-7} mol l^{-1} (25). This feature can be taken advantage of as a rapid and sensitive bioassay for coronatine.

Coronatine is derived from the name used to describe the organism first discovered to produce this toxin, P. coronafaciens ssp. atropurpurea. There is now a taxonomic distinction made between P. syringae pv. atropurpurea and pv. coronafaciens. It has been shown that pv. coronafaciens produces tabtoxin while pv. atropurpurea produces coronatine (35). Recently, coronatine production in vitro was reported in pv. glycinea (39). Nishiyama and Ezuka (40) reported P. syringae pv. morsprunorum and pv. maculicola to also produce coronatine. However, further studies (34) of several strains of each pathovar yielded negative results with P. syringae pv. maculicola, P. syringae pv. morsprunorum produced coronatine only sporadically while toxin production was found to be a general characteristic of P. syringae pv. glycinea and pv. atropurpurea.

Coronatine is produced in liquid culture optimally at $18^{o}C$. It is extracted into ethyl acetate after concentrating the supernatant at pH 6.8, then adjusting to pH 2.5. The first step in purification is partition chromatography on Sephadex LH 20 followed by preparative thin-layer chromatography on silica gel (39). High resolution mass spectrometry

(25) gives the molecular formula as $C_{18}H_{25}O_4N$, molecular weight then being 319, A partially purified toxin from P. syringae pv. glycinea was reported to inhibit a chlorophyll precursor, aminolevulonic acid (22). The inhibition of aminolevulonic acid may be related to the hypertrophy of potato cells and an indicator of the mechanism or mode of action of coronatine.

Genetics of Production

Special characteristics of bacteria are often plasmid determined. These characteristics may include 1) virulence properties such as antibiotic or toxin production, adhesion antigens, drug resistance and tumor formation ability, 2) nodulation of legumes by Rhizobium, 3) the ability to detoxify or degrade a wide variety of substances which may be toxic to other organisms (23). Plasmids are known to be involved in toxin or antibiotic production in fungi, streptomycetes and most bacteria. Whether or not plasmids contain the structural or regulatory genes for antibiotic synthesis has not been determined in every case. The relationship between plasmid content, toxigenicity and pathogenicity has been studied in two pathovars of P. syringae, pv. syringae and pv. phaseolicola.

Gonzales and Vidaver (17, 18) investigated the possible plasmid involvement in SR production with variable results. In 1979 they reported plasmid presence to be associated with SR, resistance to two bacteriophage and resistance to a bacteriocin. A strain of P. syringae pv. syringae causing holcus spot disease of maize was treated with acridine orange (a plasmid curing agent). Survivors were isolated that were unable to produce SR (SR^-) and were not pathogenic of maize plants. DNA analysis of the parent (HS 191) and the SR^- mutant revealed a 35 Mdal plasmid (PCG 131) present in the parent but absent in the SR^- strains. Investigation of other strains revealed plasmid DNA in only eight of 14 SR^+ isolates from various hosts (17). Based on this, SR probably is not plasmid determined, however, there may have been alterations in cell surface or other properties which affect the ability of the bacterium to colonize the host.

There may also have been unnoticed problems in plasmid curing or extraction procedures. The plasmid may have integrated into the chromosome, or chromosomal mutations or mutations on the plasmid went undetected. It is possible that a point mutation may go undetected in an Eco R1 digest.

In 1980, Gonzales and Vidaver (18) reported the isolation of a 35 Mdal plasmid from pathogenic, SR^+ P. syringae pv. syringae isolates from millet, almond and apricot. Comparison of restriction endonuclease digestion and agarose gel electrophoresis of the 35 Mdal plasmid from these three isolates plus pCG 131, revealed one fragment of 1.3 Mdal common to all four plasmids. This suggests some degree of relatedness of the DNA (as they contain similar restriction sites) but is not valid evidence that the 1.3 Mdal restriction fragment codes for SR. Attempts by Gonzales et al (16) at plasmid curing and retransfer and concomitant transformation of SR^- to SR^+ were not successful. Transformation occured, but did not alter the toxin phenotype.

Currier and Morgan (7), in a study of 40 strains of P. syringae pv. syringae, were not able to find any correlation between plasmids, toxin (SR or ST) production or pathogenicity. Plasmids were cured with acridine orange. Cured strains showed no alterations in SR production or pathogenicity on pear seedlings. Labelled plasmid probes did not hybridize with total DNA from their respective cured strains. This indicates that the plasmid did not integrate into the chromosome prior to acridine orange treatment.

Phaseolotoxin also appears to be chromosomally determined. Gantotti et al (14) implicated plasmid involvement in PSLT production. Through dye bouyant density gradient equilibrium centrifugation, plasmid DNA was

found in an isolate of P. syringae pv. phaseolicola, G 50. This isolate was previously subjected to ultraviolet light mutagenesis and nontoxigenic survivors were isolated. Agarose gel electrophoresis showed that G 50 Tox$^-$ contained no plasmids and the parent, G 50, contained a 22.5 Mdal plasmid. It was previously reported that this mutant was only weakly virulent on bean plants (46). Lessened virulence may have been due to an independent mutation due to the UV treatment. UV irradiation can cause thymine dimers and deletions in chromosomal and plasmid DNA (4).

Panopoulos et al (45) surveyed 18 toxigenic strains of P. syringae pv. phaseolicola and found no correlation between plasmid content and toxigenicity. Plasmids were found to fall into four molecular weight classes: from ca. 75-110 Mdal, 28-32 Mdal, 23-27 Mdal and 5.2-26. Mdal. The number of plasmids per strain varied from one to three. One of the strains surveyed was G 50 Tox$^-$ of Gantotti et al upon which the claim of plasmid determination of PSLT synthesis was made. Separate stocks of G 50 Tox$^-$ were found to contain three plasmids from three separate size classes. G 50 (parent) also contained plasmids of the same size classes. No explanation was offered for this discrepancy.

Attempts have also been reported by Panopoulos et al (45) to isolate Tox$^-$ mutants of P. syringae pv. phaseolicola by plasmid curing via acridine orange or ethidium bromide treatments. Several thousand colonies were screened and none were found to be Tox$^-$. Chemical mutagenesis by ethyl methane sulfonate (EMS) and N-methyl-N'-nitrosoguanidine (NTG) allowed the isolation of several Tox$^-$mutants. Some survivors of mutagenesis showed changes in plasmid content. There was no correlation between changes in plasmid DNA or toxigenesis. Altered plasmids were found

both in Tox^- and Tox^+ mutants. Many of the chemically mutagenized cells showed variations in the amount of toxin produced relative to the parent strains. Such variations were independent of plasmid content. This suggested the structural genes for PSLT may not be on a plasmid. There may be, however, multiple copies of the genes for toxin production present that were not accounted for (44).

Further genetic evidence against plasmid involvement was offered by Staskawicz and Panopoulos (51). They reported that spontaneous loss of pGP502 (22.5 Mdal) had been observed in G 50 Tox^+ without concomitant loss of toxigenicity. The loss of pGP502, they stated, occured spontaneously and independently of the mutational event that caused G 50 to loose the ability to produce PSLT. The possibility of integration into the chromosome by the plasmid may exist.

Evidence against toxin production being plasmid determined was presented by Jamesion et al (26). Strains of P. syringae pv. phaseolicola from a wide geographic distribution and with different levels of PSLT production (Tox^-, Tox^+, $Tox^{variable}$) were surveyed. upon comparison of the plasmid profiles, no relationship between toxigenicity and plasmid content was found. DNA hybridization experiments showed no plasmid homology between Tox^- or Tox^+ wild-type or mutagenized cells.

This wide range of evidence provides confidence enough to state that the structural genes for toxicity are not likely to be plasmid borne. Transpositional mutagenesis with resulting Tox^- phenotype has not been reported though Tn 7 is known to transpose into the chromosome (44). Theoretically, if such a mutant could be isolated, restriction endonuclease digestion followed by hybridization with a ^{32}P labelled probe,

provided such a probe could be engineered, would be a means by which to locate the PSLT gene(s).

Association with Hypersensitive Response

Phytotoxins have also been implicated in suppression of the hypersensitive response (HR) by certain hosts. The hypersensitive response is an acute, localized reaction where host plant cells die soon after inoculation with a pathogen (1). This disallows the further colonization of the host by an obligate parasite. When the pathogen is not an obligate parasite, death of the surrounding host cells does not explain why, in certain cases, the pathogen does not continue to multiply. Substances toxic to the pathogen are produced by the host and accumulate at the point of infection. These substances, phytoalexins, are generally confined to the killed cells and those adjacent to them (42).

In a series of experiments, making use of HR and phytoalexin accumulation, Patil and Gnamanickam (42, 48) were able to postulate a role for PSLT in halo blight disease of beans. Infected, resistant bean plants were able to support the growth of P. syringae pv. phaseolicola cells without concomitant PSLT production (46). They were then able to show that when leaves of resistant bean cultivars were pretreated with PSLT prior to inoculation with P. syringae pv. phaseolicola, phytoalexin accumulation was much lower than that of the nontreated controls (47). Not only did PSLT supress phytoalexin accumulation in similarly treated resistant bean seedlings, HR was also suppressed (48). The concentration of PSLT at which HR was suppressed was equal to the concentration found in infected, susceptible tissues. This indicates that PSLT, when produced

by the bacterium in the host plant, may be involved in the initial establishment of P. syringae pv. phaseolicola in susceptible hosts. PSLT is also not detectable in resistant hosts. Resistance to halo blight may be dependent on the ability of the host to supress PSLT production (48). Age of the host plant was indicated as a factor in resistance in bean plants to P. syringae pv. phaseolicola. Young tissues were not able to express HR, thus allowing production of PSLT and colonization of the host by the bacterium (41).

Part II

Toxins produced by phytopathogenic bacteria have not been considered is as great detail as those produced by microorganisms which affect man or animals. When the general characteristics of antibiotics or anti-metabolites from nonphytopathogenic bacteria are compared to phytotoxins, several similarities are immediatly apparent. In order to understand these similarities, a discussion of secondary metabolites and the production and regulation of antimetabolic substances follows.

Antibiotics

Primary and secondary metabolites

It is not clear whether or not toxins are primary metabolites, secondary metabolites or if the distinction may be made with any valididy. Primary metabolism concerns the synthesis of materials essential for the growth and survival of organisms, this includes proteins, nucleic acids, carbohydrates and coenzymes. This usually takes place during logrithmic growth, or trophophase, The pathways of primary metabolism are often very similar over a range of organisms. Secondary metabolites, in contrast, are synthesized through a great variety of pathways, though usually through a few key intermediates. Secondary metabolites are produced at slower growth rates or when a culture reaches stationary growth phase. This growth phase is often termed idiophase. Examples of these metabolites are antibiotics, alkaloids and plant growth factors. There are often great differences in secondary metabolites among closely related strains or species. An important character common to all is production at a low specific growth rate. Regulation of growth rate affects a range of biosynthetic processes.

Individual pathways are also affected by other regulatory mechanisms such as induction, catabolite regulation and end-product regulation (24, 30).

True antibiotics

Microogansims are capable of producing a wide array of metabolites which kill or inhibit the growth of other microbes. These are usually considered secondary metabolits. Distinctions are made in the literature between true antibiotics and bacteriocins which also inhibit microbial growth. Phytotoxins have been considered as antibiotics (23). The most important distinction between biocidal compounds is genetic. True antibiotics are encoded by a dozen or more genes. They are assembled by a step-wise biosynthesis, each step being catalyzed by an enzyme. Antibiotics are relatively small, not typical proteins and are active against diverse genera (23).

Most bacteriocins are proteins. They are assembled on ribosomes from the information on a single structural gene (23). Most have plasmid borne genetic determinants of production and host cell immunity. Attachment of bacteriocins to target cells requires the presence of specific cell receptors. The biospectrum of inhibition is centered around closely related (homologous) species. Commitment of the bacterium to produce a bacteriocin may lead to cell death (lethal biosynthsis) (66). The larger bacteriocins of Gram-positive bacteria and pseudomonads resemble bacteriophage components (23).

There are also two classes of "intermediates" which fall between true antibiotics and bacteriocins relevant to phytotoxins: microcins and the peptide antibiotics of bacilli and some Streptococcus and Psuedomonas

species. Microcins are a diverse class of broad-spectrum antibiotics produced by Gram-negative bacteria, mainly by strains of Escherichia coli and also by Pseudomonas aeruginosa. First described in 1976, most microcins have a molecular weight of less than 500 daltons, though one is reported to be 5,000 daltons. The larger microcins act as competitive inhibitors of methionine. Their effect is reversed by the addition of L-methionine. The addition of specific L-amino acids also reversed the effects of PSLT and tabtoxin. The microcins may be methionine analogs that act as feedback inhibitors of methionine synthesis (23).

Peptide antibiotics have several general characteristics in common (27): 1) They are generally much smaller than proteins, with a molecular weight range of 270-4,500 daltons. 2) In almost all cases, a family of closely related (analogous) peptides, rather than a single entity, is synthesized by an organism. 3) Most are composed entirely of amino acids. Some may contain substititutions or other constituents. 4) Frequently, unique, nonprotein amino acids are components. Reported so far have been D-amino acids, β-amino acids, dehydroamino acids and sulfer-containing amino acids. 5) Most of the peptides are cyclic, some are linear, often containing unusual linkages or arrangements of amino acids. 6) They are generally resistant to hydrolysis by peptidases and proteases of animal or plant origin. However, there are several antibiotics known to be susceptible to proteases. 7) Synthesis is initiated after the completion of trophophase. Industrial fermentations, however, take place during rapid growth and continue for several days. This is achieved via manipulation of carbon and nitrogen metabolism, phosphate level and induction mechanisms. These are commonly implemented means of regulating industrial fermentations (8).

Antibiotic synthesis

Synthesis of the peptide antibiotics of bacilli, gramicidin S and tyrocidin produced by Bacillus brevis and bacitracin A produced by B. licheniformis have been well studied due to their economic importance. Nonribosomal peptide bond formation is catalyzed by an antibiotic synthetase of two or three subunits: light and heavy for gramicidin S (50), light; intermediate and heavy for tyrocidin; A, B and C for bacitracin A. Peptide chain synthesis proceeds by sequential addition of amino acid residues to a growing chain from the N-terminus. Each amino acid is bound through a thioester bond to the enzyme at a spatially distinct site on one or more of the subunits. A separate protein exists for each amino acid. The peptide chain is handed from one site to the amino acid bound at the next site of the same or adjacent subunit. Racemization of phenylalanine residues in gramicidin S synthesis seems to occur at the binding site. Cyclization of tyrocidin and bacitracin A then occurs as does the head-to-tail joining of the two pentapeptides of gramicidin S (29). The mechanisms of cyclization and release are not yet known. There is also a small protein on each of the two subunits of tyrocidin that binds phosphopantotheine, which is required for peptide bond formation (29). Syringomycin may be produced on a similar subunit type mechanism (23) though this has yet to be documented.

Control of synthesis

The structural genes for antibiotic synthesis can be located on plasmids, though, for the most part, they are chromosomal. Frequently, the regulatory genes which control expression of the genetic information

are plasmid borne. At high growth rates, antibiotic genes are not expressed. This suggests the antibiotic synthetases are not formed or, their activity is inhibited (30).

The appearance of antibiotic synthetases occurs at late growth stages. This could be due to interference of transcription of the genetic information fron DNA to mRNA. Also translation of the information from mRNA to the antibiotic synthetase could be disrupted. It is not known exactly at which level repression occurs in the majority of antibiotic syntheses (30). Available evidence shows either transcription or tr a nslation may be interfered with, depending of the organism and particular antibiotic being synthesized.

Synthetase action can be inhibited during rapid growth. Several β-lactam antibiotics are made after growth is completed. Resting cell studies show high levels of β-lactam synthetases present during trophophase (30) (cycloheximide was added to prevent protein synthesis by the resting cells).

Some mechanisms of control of antibiotic synthetases function at more than one level of control. Phosphate control of candicidin synthesis by S. griseus is exerted at two levels. At the first level, biosynthesis is repressed until phosphate is depleted from the broth. Candicidin synthesis is inhibited again by phosphate after the synthetase has been formed.

Branched pathways are commom in several systems, including the synthesis of several antibiotics. The early part of a pathway is common to two or several compounds, it then branches to the synthesis of a primary metabolite and to the synthesis of a secondary metabolite. Primary end-

product feedback inhibition to the common part of the pathway inhibits antibiotic synthesis (30).

Effectors and mechanisms of synthesis

Two models have been proposed for the control and initiation of antibiotic biosynthesis (30): 1) A small molecule represses or inhibits the formation or action of antibiotic synthetases. This small molecule must be depleted before antibiotic synthesis can occur. This fits in with experimental data on carbon catabolite regulation, nitrogen metabolite regulation and phosphate control (9). 2) An inducer or activator must be synthesized by the organism or added to it in order to initiate synthesis.

Glucose is usually an excellent carbon source for growth, however, it interferes with the biosynthesis of many antibiotics (27). In fermentations, poly- or oligosaccharides as carbon sources are often found to produce higher yields of antibiotics. When a medium contains both glucose and a slowly utilized carbon source, glucose is used first without concomitant antibiotic formation. The second carbon source is then used during antibiotic synthesis. Glucose has been reported in a few cases to act as a repressor of a specific enzyme synthetase. Also playing a role in regulation is decreased pH or depletion of dissolved oxygen. Decreases in pH are caused by undissociated forms of acetic and pyruvic acid that accumulate when cultures are grown in high glucose concentrations (27, 30).

Cyclic adenosine 3,5-monophosphate (cAMP) may be involved in carbon catabolite repression of inducible catabolic enzymes in some systems as a positive effector. High glucose concentrations inhibit The activity

of adenyl cyclase, decreasing the intercellular level of cAMP. cAMP interacts with cAMP receptor protein, this complex then binds to the promotor sites of the operons coding for inducible enzymes, thus activating gene transcription. cAMP has not been directly linked to carbon catabolite regulation in antibiotic synthesis. Indirect evidence suggests antibiotic synthetases might be turned off by high levels of cAMP (30). cAMP is probably more closely related to phosphate regulation than to carbon catabolite regulation.

Ammonia, or another readily used nitrogen source, may act as a regulatory mechanism in some bacteria, yeasts and molds (9). Enzymes involved in the use of other nitrogen sources are repressed: included are: nitrite reductase, nitrate reductase, NAD-dependent glutamate dehydrogenase, arginase, ornithine transaminase, extracellular protease, acetamidase, threonine dehydratase and allantoinase, among others. Glutamine synthetase functions both in glutamine formation and in the regulation of enzymes involved in assimilation of nitrogen compounds. Few studies have been reported on nitrogen metabolite regulation. Slowly used nitrogen sources, such as slowly metabolized amino acids, yield higher amounts of antibiotics during fermentaions (30).

In many fermentations, phosphate is the growth limiting factor. Phosphate is usually depleted before the onset of antibiotic synthesis. Phosphate can also inhibit certain syntheses once initiated. Industrial fermentaions of peptide antibiotics, polyenemacrolides, tetracyclines and complex antibiotics are carried out a growth limiting concentration of inorganic phosphate (<10 mM) (9). The pathways of antibiotic synthesis are numerous, phosphate may be a common regulatory effector that acts on

the various pathways, or it may be a component in several mechanisms. Phosphate addition to an antibiotic fermentation causes a reversal after several hours from nongrowing, antibiotic producing cells to growing, non-producing cells (30).

It is not yet known whether intracellular orthophosphate is the effector itself or a regulator of the level of another intracellular effector controlling antibiotic synthesis. Also under investigation is whether ATP concentration or energy charge of the cell is the parameter of enzyme activities.

Phosphatase regulation is also important during antibiotic synthsis. Microbial phosphatases that cleave phosphorylated intermediates are usually regulated by inorganic phosphate via repression or feedback inhibition. Many antibiotic intermediates are phosphorylated while the end product is not. Most phosphatases functioning in antibiotic biosyntheses are inhibited by high levels of phosphate (30).

The phenomenon of enzyme induction is often a regulatory mechanism controlling primary metabolism. Few studies have been carried out to determine the extent of induction in control of antibiotic synthesis and expression. It must also be determined if the stimulatory effect is true induction or a precursor effect. Inducers are considered to be compounds which stimulate antibiotic biosynthesis when added during the growth phase before idiophase, but not when added to idiophase cells in which protein synthesis has been inhibited by the addition of an inhibitor. These compounds should be replaceable by a nonprecursor analog. Precursors, then, stimulate antibiotic synthesis when added during idiophase, even when protein synthesis has been blocked. This phenomenon has been reported in streptomycin biosynthesis by S. griseus, cephalo-

sporin C biosynthesis by Cephalosporium acremonium and other β-lactam antibiotics (30).

Cessation of synthesis

The duration of antibiotic production differs among producing organisms and environmental conditions (27, 30). After expression and/or formation of the antibiotic synthetases, production increases and is linear. This linear period can be as short as four hours or, through manipulation of the cultural conditions in the fermentation chamber, can last as long as 300 hours. Using fedbatch systems, penicillin production may last up to 10 days.

Loss of viable, producing cells is not the cause of cessation of antibiotic synthesis. As long as the carbon source is not depleted, lysis of no longer viable cells does not occur. There are several possible reasons for cessation of production, again, they are strain and antibiotic dependent. The least likely explanation is the irreversible decay of one or more synthetases in the pathway. More likely reasons are the feedback effect of the accumulated antibiotic, or the depletion of precursor molecules (27).

Comparisons of antibiotics and phytotoxins

The exact processes involved in the biosynthesis of most phytotoxins are still unknown. Several factors contribute to this lack of information (10): 1) the chemical structures of many toxins have not been completly identified, 2) many are difficult to purify, 3) radiolabeling is difficult as most toxins make up only a small fraction of C or N in a culture, in some cases, ^{35}S or ^{32}P can be used when those atoms are present in the toxin

molecule, 4) many toxins are not stable *in vitro* and/or *in vivo*, 5) toxins are active at low concentrations, $<10^{-6}$ M, and 6) artifacts are usually created when toxins are introduced into a plant.

Even with such difficulties, some speculation can be made on the biosynthetic pathways of a few phytotoxins. The low molecular weight of many bacterial toxins suggests involvement of soluble enzymes that have recognition sites for two or three point attachment of small molecules. Some peptide toxins might be synthesized via step-wise assembly on individual enzymes much like the synthesis of peptide antibiotics.

Closer inspection of the available data on phytotoxins suggests that not only may the biosynthesis of phytotoxins resemble peptide antibiotics but they may have other characters in common as well (Tables 2 and 3). Their molecular weights reported so far are within the range of those reported for peptide antibiotics and microcins. Pathovars of *P. syringae* produce phytotoxin analogs with substituted amino acids, serine for alanine in PSLT and for threonine in tabtoxin. These analogs possess equal biologic activity as the original compound. Bacilli will preferentially incorporate exogenously supplied amino acids during antibiotic synthesis in culture and in cell-free synthesis (27). It is possible substitutions would occur during the synthesis of phytotoxins. Rudolph et al (54) found that by varying the amount and combinations of various amino acids to *P. syringae* pv. *phaseolicola* culture medium, toxin was produced at earlier or later stages of growth, depending on the amino acid added. This may indicate the formation of PSLT analogs not yet described. Most of the phytotoxins described so far contain unusual or nonprotein amino acids along with substituted amino acids: ornithine, tabtoxinine or substituted lysine in SR and the N-phosphosulphamyl substituted ornithine

in PSLT. SR, ST and PSLT are linear, not unlike the majority of the peptide antibiotics. The peptide backbone of PSLT and its serine analog are susceptible to enzymatic hydrolysis by carboxypeptidase and plant enzymes, though the active moiety, (N^{δ}-phosphosulphamyl)ornithine is not (36). The tabtoxins are subject to hydrolysis by plant enzymes. In both cases, hydrolysis is required for formation of the active <u>in vivo</u> toxin. Both PSLT and phosphosulphamylornithine are active, however, phosphosulphamyl ornithine is found to a much greater extent in plant tissues.

Metabolism

Metabolic regulation of toxin production has only been studied is relation to growth curves and cultural conditions (56). Most studies have shown variability in toxin production between culture conditions and among isolates. If such variability is due to presence or absence of required growth factors or an innate quality of the organism is not known. Perhaps active or inactive analogs or isomers are produced at certain times that are not detected via the bioassay being used to monitor toxin production

Preliminary studies show wide varieties of phaseolotoxin yield can be obtained depending of the cultural conditions of P. syringae pv. phaseolicola. Glycerol, glutamate, cysteine and yeast extract seem to stimulate production. Phosphate concentration showed no effect, perhaps ruling out the possibility of cyclic AMP or ATP as modulators. Cysteine and glutamate may be indirect precursors of the PSLT molecule. The addition of yeast extract possibly spares precursor molecules. High concentrations of glucose may cause carbon catabolite repression as PSLT production is lower at high concentrations, slowly metabolized carbon sources provide higher PSLT production (54). PSLT is produced in detectable amounts during all growth stages. Maximum production occurs near the end of log phase, when the carbon source is depleted (54).

In the case of SR, glucose and caseamino acids stimulate production, thus carbon or nitrogen metabolites probably are not regulators. High phosphate concentration represses SR synthesis, ATP may then be acting as an effector molecule.

Once produced, several fates await a toxin in diseased plant tissue.

If the toxin is already in its biologically active form, it may interact with the host at the primary site(s) of action. The toxin may diffuse away or be actively taken up by the host and translocated away through the phloem or xylem, never reaching its active site. The toxin may be modified chemically or biochemically (10). The enzymes responsible for modification may be of host plant origin, as is the case in PSLT cleavage to (N^{δ}-phosphosulphamyl)ornithine, the active toxin *in vivo*. Both PSLT and (N^{δ}-phosphosulphamyl)ornithine are biologically active and inhibit OCT, the target (36). Uchytil and Durbin (69) showed that both plant and bacterial enzymes were responsible for the hydrolysis of tabtoxins *in vitro* and *in vivo*. Highly purified tabtoxin had little or no effect of purified GS, the site of action. ^{14}C-labelled tabtoxinine-β-lactam could be isolated from healthy tobacco leaves injected with ^{14}C tabtoxins after a short incubation period. This re-isolated tabtoxinine-β-lactam is active against GS. Thus it seems hydrolysis is necessary for biological activity (10).

Of the modes of action of toxins thus far uncovered, most target a primary metabolic pathway of the host. This then raises the question why does the producing organism not suffer any of the deletarious effects of its own toxin? There are several possible mechanisms bacteria employ to avoid suicide (8): 1) permeability modifications, 2) internal degradation or modifications of the toxin, 3) allosteric feedback control of toxin synthesis, 4) altered affinity of the target(s) for the toxin, 5) cellular compartmentalization, 6) production of inactivating metabolites, or 7) a combination of the above.

Tabtoxins or tabtoxinine-β-lactam are not taken up by isolates of *P. syringae* pv. *tabaci* rendering them insensitive to their own toxin.

Isolates of P. angulata (nomen dubium), which is considered a Tox^- form of P. syringae pv. tabaci, rapidly take up tabtoxins and tabtoxinine-β-lactam (10). Mutants resistant to tabtoxins or tabtoxinine-β-lactam of P. angulata do not take up the compound. GS from all sources was inhibited by tabtoxins and tabtoxinine-β-lactam. Resistance could be explained by the inability of the toxin to reach its active site rather than GS being directly involved in resistance (10).

Spontaneous chemical modification occurs in the tabtoxins as well as the biochemical changes described. The unstable β-lactam undergoes an interal shift to the more thermodynamically stable, though biologically inactive, δ-lactam form. The requirements for the active site of GS can no longer be met with the δ-lactam form. This shift is dependent on pH and temperature. It follows a first-order decay curve with time.

Staskawicz et al (62) showed P. syringae pv. phaseolicola produces two forms of the target enzyme of PSLT, OCT. One form resistant to PSLT is produced at 18°C, which is optimal for toxin production. At higher temperatures where PSLT is not produced, the form of OCT produced is sensitive to PSLT.

Thus, when studying toxins in vivo two complete metabolic systems must be accounted for. When in the presence of each other, plant and bacterial systems often cause modifications not seen when studied separately. It is therefore imperitive to have a full understanding of the pathogen and the host before initiating a combined study.

Concluding Remarks

The metabolites of fluorescent pseudomonads have been studied since the beginning of the twentieth century. During the past 80 years, innumerable compounds have been isolated and/or analyzed. Only two antibiotics produced by pseudomonads are available for practical application (pyocyanine, pyrrolnitrin) (29). There is little information on the biosynthesis or regulation of secondary metabolites from this group.

Pseudomonads represent the major group of nondifferentiating microorganisms which produce antimetabolites (29). The increasing knowledge on the biochemistry and genetics plus the nutritional and environmental diversity of fluorescent pseudomonads will soon make them indispensible as a tool for studying secondary metabolism without the interference of genetically programmed cellular differentiation (29). This information could be applied to antibiotic production by bacilli, much as the converse is done now.

Further studies of the low molecular weight phytotoxins of P. syringae pathovars will undoubtedly increase the existing body of knowledge on metabolites produced by pseudomonads. They are also of great practical significance, as they are responsible for disease symptoms on host plants (64) by invading bacteria. Increased investigations of these compounds may ulitmately lead to the control of certain plant diseases.

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Table 1. Known Phytotoxins of Pathovars of *Pseudomonas syringae*.

pv. *tabaci*	Tabtoxin[a], (2-serine) tabtoxin[b]
pv. *coronafaciens*	Tabtoxin[a], (2-serine) tabtoxin[b]
pv. *garcae*	Tabtoxin[c]
pv. *phaseolicola*	Phaseolotoxin[d], (2-serine) phaseolotoxin[e]
pv. *atropurpurea*	Coronatine[c]
pv. *glycinea*	Coronatine[f]
pv. *syringae*	
(stone fruit, pear, grasses)	Syringomycin[g]
(citrus)	Syringotoxin[h]
pv. *tagetis*	Tagetitoxin[i]
pv. *tomato*	Ammonia (unconfirmed)[j]
pv. *mori*	Uncharacterized[c]
pv. *morsprunorum*	Uncharacterized[c]
pv. *maculicola*	Uncharacterized[k]

[a]Stewart (63).

[b]Taylor et al (67).

[c]Mitchell (34).

[d]Mitchell (32).

[e]Mitchell (33).

[f]Mitchell and Young (39).

[g]Sinden et al (58).

[h]Gonzales et al (19).

[i]Mitchell and Durbin (37).

[j]Bashan et al (3).

[k]Gasson (15).

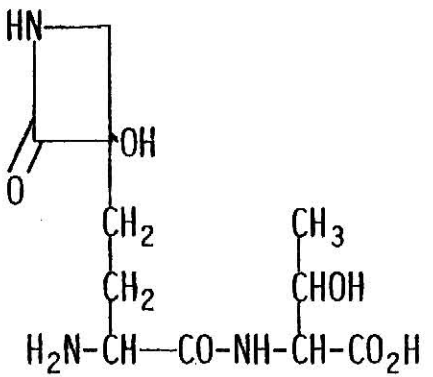
Table 2. Comparison of General Characteristics of Antibiotics, Bacteriocins, and the Phytotoxins of Pathovars of Pseudomonas syringae.

Antibiotics	Bacteriocins	Phytotoxins
assembled via stepwise biosynthesis, catalyzed by specific enzymes	assembled on ribosomes	not known, probably stepwise mechanism
relatively small	large, some may resemble bacteriophage components	relatively small
active against diverse genera	active against homologous species	active against diverse genera
may or may not have plasmid borne genetic determinants of production, usually are chromosomal, regulatory genes usually extra-chromosomal	usually plasmid borne genetic determinants or production	probably chromosomal determinants of production
various mechanisms of host cell immunity	usually plasmid borne genes for host cell immunity	various mechanisms of host cell immunity
no specific receptor sites	specific receptor sites on host cell	no specific receptor sites
produced late in growth, related to differentiation, no evidence for lethal biosynthesis	may lead to lethal biosynthesis	no evidence for lethal biosynthesis, probably late growth stage for production

Table 3. Comparison of Selected Characteristics of Peptide Antibiotics, Microcins and the Phytotoxins of Pathovars of *Pseudomonas syringae*.

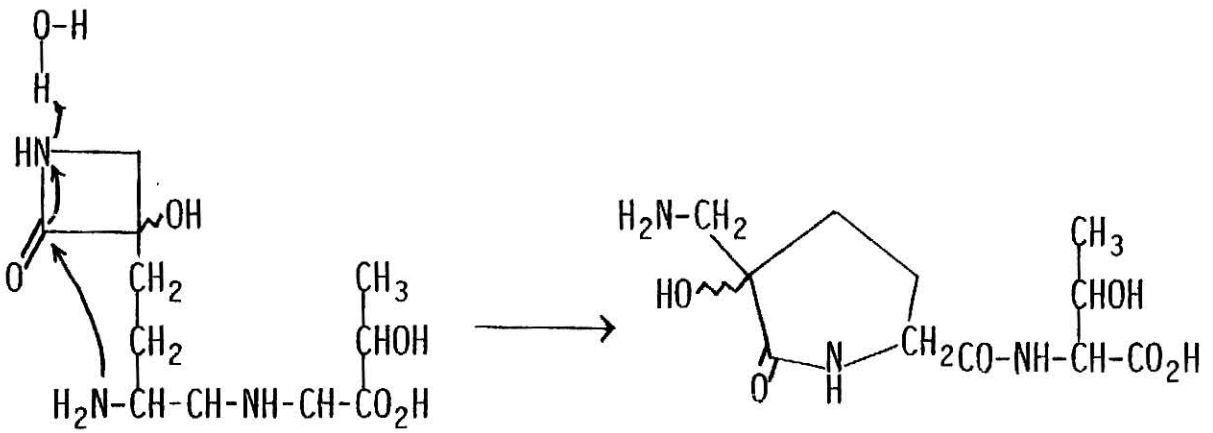
Peptide Antibiotics	Microcins	Phytotoxins
mol wt 270-4,000	mol wt < 500	mol wt < 1,000
peptides or single amino acids	oligopeptides	commonly peptides or amino acids
wide variety of mechanisms	some are competitive inhibitors of methionine, addition of specific amino acid reverse effects	competitive enzyme or amino acid inhibitors, addition of specific amino acids reverses effects
family of analogs synthesized rather than a single entity	probably substitutions in the peptide are made	analogs (active and inactive) are known
substituted amino acids common	unknown	substituted amino acids occur
unique, nonprotein amino acids common	unknown	unique, nonprotein amino acids occur
cyclic, linear compounds and unusual linkages common	linear peptides	cyclic, linear compounds occur
generally are resistant to hydrolysis by proteases of animal or plant origin	larger microcins are protease susceptible, smaller ones are resistant	some peptide bonds susceptible to enzyme hydrolysis, active site not susceptible
produced late in growth (idiophase)	unknown	unknown, probably late stages of growth

Figure 1.



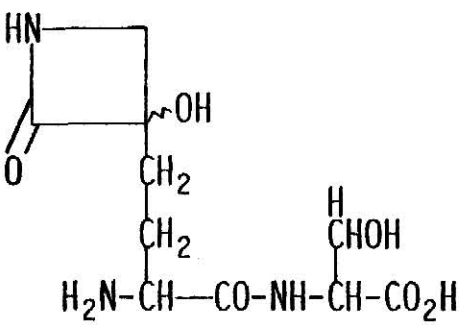
STRUCTURE OF TABTOXIN (STEWART, 1971)

Figure 2.



INTRAMOLECULAR ISOMERIZATION OF TABTOXIN TO ISOTABTOXIN (MITCHELL, 1981).

Figure 3.



B) STRUCTURE OF (2-SERINE)TABTOXIN
(TAYLOR ET AL., 1972)

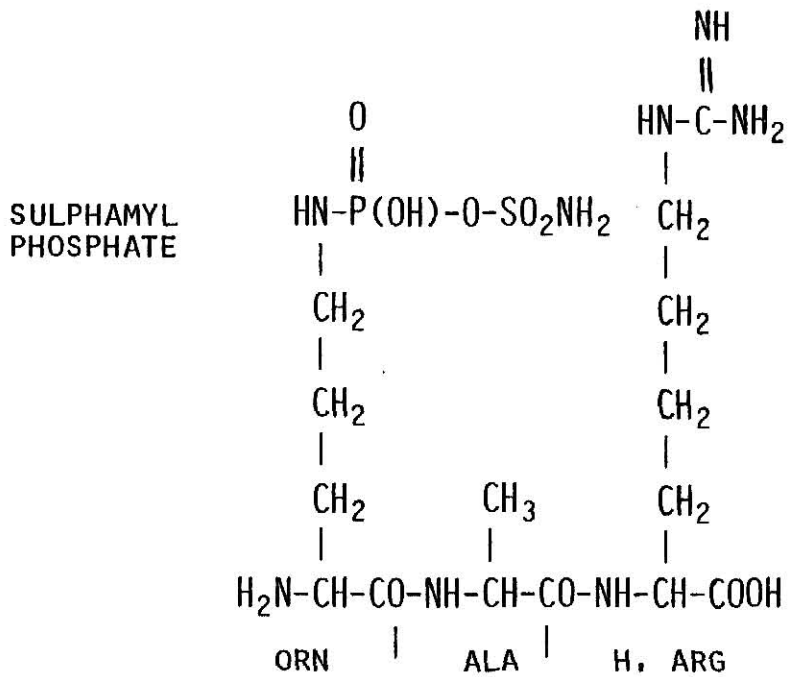
Figure 4.

A) Composition structure of Syringomycin

B) Composition of Syringotoxin

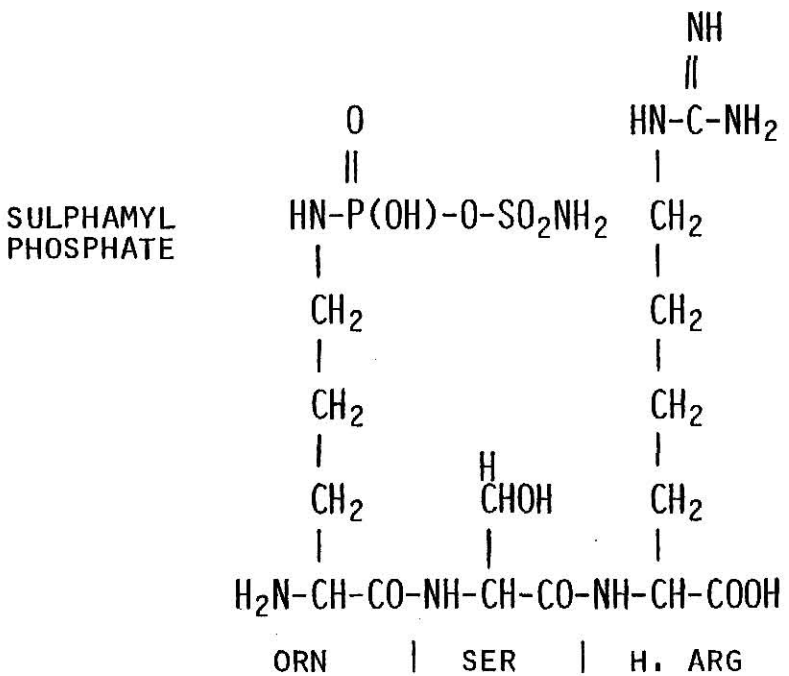
	Molar Ratio
Serine	2
Phenylalanine	1
(Lysine)	2
Arginine	1
Threonine	1
Serine	1
Glycine	1
Ornithine	1
Unidentified basic component	

Figure 5.



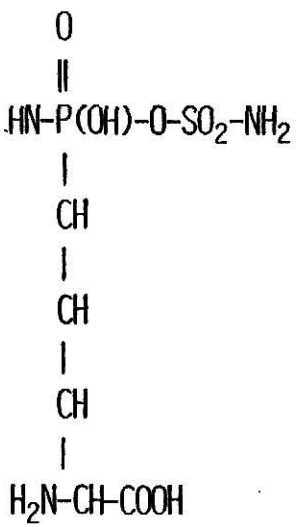
STRUCTURE OF (N^{δ}-PHOSPHOSULPHAMYL)ORNITHYLALANYLHOMOARGININE, PHASEOLOTOXIN (MITCHELL, 1976)

Figure 6.



STRUCTURE OF (2-SERINE)PHASEOLOTOXIN (MITCHELL, 1978)

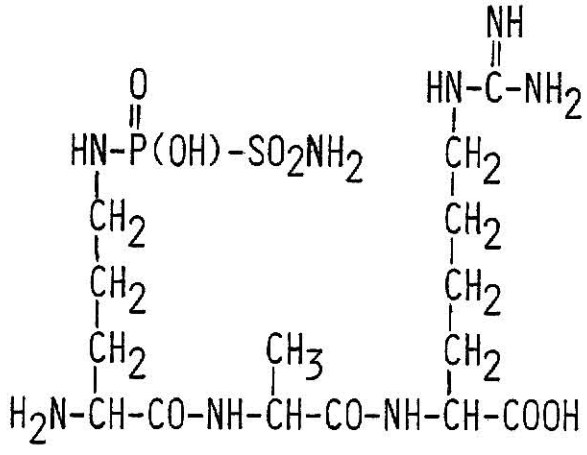
Figure 7.



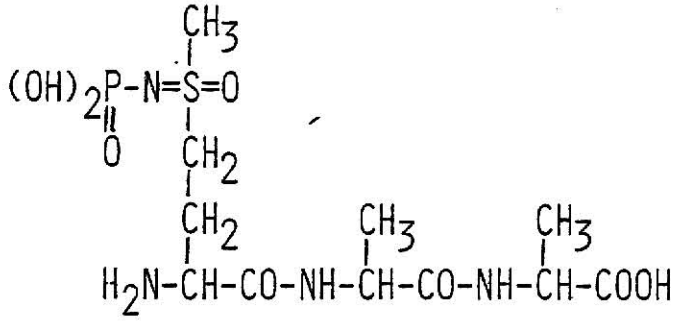
STRUCTURE OF PHOSPHOSULPHAMYLORNITHINE (PSORN)
(MITCHELL AND BIELESKI, 1977)

Figure 8.

A)

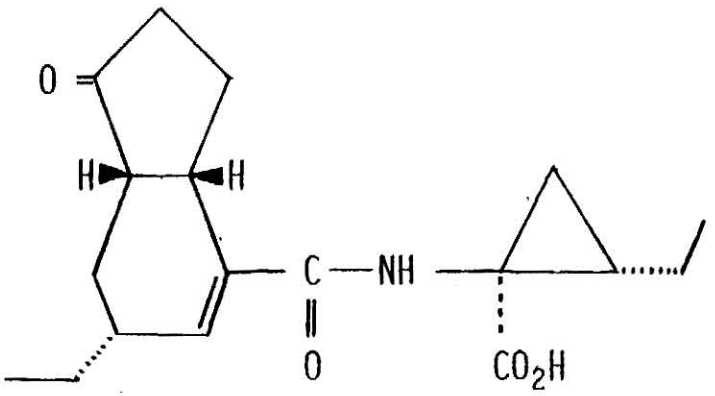


B)



COMPARISON OF PHASEOLOTOXIN, (A), (N^{δ}-PHOSPHOSULPHAMYL) ORNITHYLALANYLHOMOARGININE, AND (B), L-(N^{5}-PHOSPHONO) METHIONINE-S-SULFOXIMINYL-L-ALANYL-L-ALANINE FROM AN UNCLASSIFIED STREPTOMYCES (PREUSS ET. AL., 1973 AND MITCHELL, 1976).

Figure 9.



STRUCTURE OF CORONATINE (ICHIHARA ET AL., 1977)

PHYTOTOXINS PRODUCED BY PATHOVARS OF *PSEUDOMONAS SYRINGAE*

by

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B.S., Eastern Illinois University, 1980

AN ABSTRACT OF A MASTER'S REPORT

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Manhattan, Kansas

1983

ABSTRACT

Many of the disease symptoms and loss due to disease in plants are caused by phytotoxic compounds. The phytotoxins of various pathovars of Pseudomonas syringae can cause severe disease symptoms. Recently, more emphasis has been placed on their biochemistry, structure, mode of action, biosynthesis and possible role in disease development. Phytotoxins that have been completely or partially characterized are tabtoxin from P. syringae pv. tabaci, syringomycin and syringotoxin from P. syringae pv. syringae, phaseolotoxin from P. syringae pv. phaseolicola, tagetitoxin from P. syringae pv. tagetis and coronatine from P. syringae pv. atropurpurea and pv. glycinea. Several phytotoxins bear striking similarities to antibiotics produced by bacilli and microcins from Escherichia coli and pseudomonads. Information from peptide antibiotic studies can, in some cases, be applied to phytopathogenic bacteria. Toxin producing pseudomonads may be useful in studying the mechanism and control of antibiotic synthesis without the interference of cellular differentiation which occurs in bacilli, fungi and actinomycetes. This combined knowledge may help in uncovering the role of toxins in disease and from these, means of controlling disease.

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