Production and regulation of potato-scab-inducing phytotoxins by *Streptomyces scabies*

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Phytotoxins with potato-scab-inducing activity were produced by the pathogenic *Streptomyces scabies* strain RB2 when grown on oatmeal agar medium or in oatmeal broth medium. Five compounds isolated from cell filtrates were identified as thaxtomin compounds 1–5, previously reported as produced by *S. scabies* grown on potato slices. The optimum temperature for phytotoxin production in oatmeal broth medium was 28 °C. Production of thaxtomin A, the major product, was repressed at least 130-fold when *S. scabies* RB2 was grown in oatmeal broth medium supplemented with 0.5% glucose. Thaxtomin A production was also repressed by tryptophan and tyrosine, precursors of which may be involved in feedback inhibition of early steps in biosynthesis. Phytotoxins were secreted by the organism when the cells reached late exponential to early stationary phases of culture growth. The time during growth at which each thaxtomin compound was produced suggests a pathway for the latter steps in thaxtomin biosynthesis.

Introduction

Streptomyces scabies (Lambert & Loria, 1989) is the causative agent of scab disease on a variety of underground vegetables including radish, beet and potato (Davis & Garner, 1978). Potato scab adversely affects tuber quality and market value, but not yield (Hooker, 1981; Slack, 1992). Scab lesions vary in colour from brown to black. The morphology of the scab lesion can range in appearance from small raised cork-like tissue around the lenticel to large deep sunken pits up to 7 mm in depth (Archuleta & Easton, 1981; Davis & Garner, 1978; Hooker, 1981).

S. scabies infects the tuber through an immature lenticel or a wound site. In response to invasion by the bacterium, suberin is synthesized at the site of infection by cells which lie a few cell layers below the surface of the tuber (Davis & Garner, 1978; Hooker, 1981). Suberin is the natural protective layer of the tuber, composed of long chain fatty acids, dicarboxylic acids, fatty alcohols and phenolic compounds (Kolattukudy, 1980, 1981), and is thought to function as a barrier to both moisture loss and microbial attack (Kolattukudy, 1984).

A class of phytotoxins, thaxtomin, has been identified

which may be involved in infection of the potato by *S. scabies* (Lawrence *et al.*, 1990). Five thaxtomins, designated compounds 1 to 5, were isolated and purified from a pathogenic strain of *S. scabies* grown on surface-sterilized potato slices (King *et al.*, 1992). Compounds 1 and 4 were previously identified as thaxtomin A and B, respectively (King *et al.*, 1989). Thaxtomins were shown to be unique 4-nitroindol-3-yl-containing 2,5 dioxopiperazines (King *et al.*, 1989, 1992). These purified compounds induce the formation of scab-like lesions when applied to the potato tuber (King *et al.*, 1992), but the mode of action of these compounds is unknown. Analysis of 19 *S. scabies* isolates demonstrated that pathogenicity was positively correlated with the isolates' ability to produce thaxtomin on potato slices (King *et al.*, 1991), emphasizing the potential importance of these compounds in scab disease.

To study the synthesis of phytotoxins associated with potato scab disease, the ability of *S. scabies* to produce phytotoxins in a synthetic medium was investigated. Phytotoxins were produced by *S. scabies* when grown in an oatmeal medium, and the structures of these compounds were determined. Several factors which affected thaxtomin production were also investigated. The ability to produce thaxtomins in liquid culture has facilitated the production and purification of these compounds and will be valuable in elucidating the pathway for phytotoxin biosynthesis.

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Methods

Bacterial strain and growth. *Streptomyces scabies* strain RB2 is a pathogenic isolate obtained from a deep-pitted scab lesion on the potato cultivar Russet Burbank grown in Becker, MN, USA. *S. scabies* was maintained on oatmeal agar medium (Shirling & Gottlieb, 1966) and grown at 30 °C. The bacterium was tested for phytotoxin production after growth on MM agar (Hopwood *et al.*, 1985), NMM agar (as in Hopwood *et al.*, 1985, but with the addition of 1.5% agar, w/v, and lacking PEG), Czapek Solution agar (Difco Laboratories) and oatmeal agar. To test whether a potato-specific compound might be necessary to induce phytotoxin production, 4% (final concentration, v/v) potato-dextrose broth (PDB) was added to MM agar and Czapek Solution agar. PDB was made by autoclaving 200 g sliced potato per litre of water for 20 min, straining, and adding 20 g dextrose l⁻¹.

Oatmeal broth medium was prepared by boiling 20 g oatmeal per litre of water for 20 min. Particulate matter was removed by filtration through cheesecloth, centrifugation at 10000 *g* for 10 min, and filtration through Whatman no. 1 paper. Trace elements were added to the oatmeal broth to a final concentration (per litre) of 80 µg ZnCl₂, 400 µg FeCl₃·6H₂O, 20 µg CuCl₂·2H₂O, 20 µg MnCl₂·4H₂O, 20 µg Na₂B₄O₇·10H₂O and 20 µg (NH₄)₆Mo₇O₂₄·4H₂O. Growth in oatmeal broth medium was monitored by following the culture OD₆₅₀, using uninoculated medium as a blank. Dry cell weight measurements of culture samples were also used to monitor growth and correlated well with the OD₆₅₀ measurements.

Potato mini-tuber assays. Two methods were used to generate potato mini-tubers for pathogenicity and scab lesion assays. *Solanum tuberosum* var. Kennebec mother plants were grown from seed pieces in the greenhouse until flowering. Leaf bud cuttings were taken from the mature plant and placed in five-inch pots of steam-sterilized sand (Lauer, 1977). Kennebec minitubers formed within 3–4 weeks. These minitubers were inoculated directly with *S. scabies* spores harvested from oatmeal agar medium with sterile water. Phytotoxin preparations were applied to 6 mm sterile filter paper discs and air-dried. The sample discs were applied directly to the tubers.

In the second method, sterile plants of *Solanum tuberosum* var. Pontiac were cultured in test tubes in MS basal medium with 0.4% agar (Murashige & Skoog, 1962) and grown in the laboratory under Gro-lux lights (GTE Sylvania). When the plants reached 5 cm in height they were transferred to trays containing steam-sterilized sand. On days 7 and 21 the plants were fertilized with 1.0 g of a commercial 20:20:20 (N:P:K) soluble fertilizer per 30 plants. After five weeks of growth, five Pontiac plants with attached minitubers were transplanted to a 12.5 cm diam. clay pot containing steam-sterilized sand. During transplanting, the minitubers were treated either with spores of *S. scabies* or with 20 µl phytotoxin dissolved in acetone. Acetone preparations were allowed to dry on the tuber surface before covering with sand. Scab lesions on the potato tuber induced by phytotoxin application were apparent after 24 h incubation. Lesions induced by spores were visible after 7–10 d.

Phytotoxin isolation. Phytotoxins were extracted from *S. scabies* grown on agar medium or potato slices and purified by a modification of the procedure of Lawrence *et al.* (1990). Agar media plates were inoculated with spores of *S. scabies* RB2 and incubated at 30 °C for 10 d. Surface-sterilized potato slices were inoculated and incubated at room temperature for 10 d. The bacteria, including the agar medium or potato slice, were homogenized in acetone using a Waring blender. The cell debris and medium were removed by filtration, and the acetone-soluble material was dried using a rotary evaporator. Phytotoxins were dissolved in water and extracted three times with an equal vol. chloroform. The chloroform fractions were collected and dried. Phytotoxin was dissolved in acetone to test for biological activity and for further purification.

Phytotoxins were also extracted from cultures of *S. scabies* RB2 grown in oatmeal broth medium. In initial experiments, the cell pellet harvested from the oatmeal broth medium was extracted with an equal vol. acetone. The acetone was evaporated, and the sample was resuspended in water. Both the cell pellet and cell filtrate samples were extracted three times with equal vols chloroform. The chloroform fractions from the cell pellet or cell filtrate were dried under vacuum using a rotary evaporator. Phytotoxin preparations were resuspended in chloroform for analysis by TLC or in acetone for testing scab-lesion-inducing activity.

To investigate the timing of production of these compounds, a 2 l flask containing 400 ml oatmeal broth medium was inoculated with *S. scabies* RB2 spores. The culture was incubated at 30 °C on a rotary shaker. Springs were placed in the flasks to increase aeration. The flask was capped with two milk filters (Kendal Agricultural Products) to aid in oxygen exchange, attached with a rubber band. Bacterial growth was measured as OD₆₅₀. At various times during growth, 25 ml samples were taken, the cells were removed by filtration through Whatman no. 1 paper, and the cell filtrate was extracted three times with an equal vol. chloroform. The chloroform was evaporated, and the samples were solubilized in chloroform for TLC analysis.

Phytotoxins were purified by silica gel TLC in chloroform/methanol (9:1, by vol.) and by reverse phase C18 TLC in acetone/H₂O (3:2, by vol.) as described in Lawrence *et al.* (1990). The molecular masses of the five thaxtomin compounds were determined by Fast Atom Bombardment (FAB) mass spectroscopy using a Kratos MS-25 mass spectrometer. NMR spectra were measured for samples dissolved in deuterated methanol with a Nicolet NT-300 WB spectrometer at 300 MHz. The spectrum of compound 3 was also measured in deuterated chloroform.

Quantification of thaxtomin A production. Samples were analysed and quantified by HPLC using a Hewlett Packard 1090 with an Alltech Econosil C18 column (10 µm particle size, 4.6 × 250 mm). Phytotoxins were eluted with a 25–50% acetonitrile gradient over 20 min and monitored as A₃₈₀. Purified thaxtomin A was weighed and a standard solution of 1.0 mg ml⁻¹ was used to establish a standard curve relating µg thaxtomin A to peak area measured at 380 nm by HPLC. Final amounts of thaxtomin A produced under experimental conditions were reported as mg thaxtomin A (g cell dry wt)⁻¹.

Results

Phytotoxin production on synthetic agar media

Six synthetic agar media were chosen to test for phytotoxin production by *S. scabies*. As a positive control for phytotoxin production, *S. scabies* was also grown on surface-sterilized potato slices as reported by Lawrence *et al.* (1990). Agar media and potato slices were inoculated with *S. scabies* and incubated for 10 d. Phytotoxin was extracted from the media and bacterial lawns and tested for its ability to elicit lesion formation on mini-tubers of *Solanum tuberosum* var. Kennebec. The results of the lesion testing (Table 1) demonstrated that scab-inducing compounds were produced when *S. scabies* was grown on potato slices or on oatmeal agar medium, but not on the other media used for growth. The assay for scab-inducing activity using Pontiac minitubers yielded similar results (data not shown).

The scab lesions induced by phytotoxins isolated from *S. scabies* RB2 grown on oatmeal agar were similar to

Table 1. Lesion formation on Kennebec mini-tubers induced by phytotoxins

Treatment*	Severity of lesions formed† (no. of mini-tubers)			
	—	+	++	+++
Oatmeal agar	1	2	2	15
Czapek agar	20	0	0	0
Czapek agar + PDB	20	0	0	0
NMM agar	20	0	0	0
MM agar	20	0	0	0
MM agar + PDB	20	0	0	0
Potato slices	5	9	5	1
Acetone	20	0	0	0
Uninoculated oatmeal agar	20	0	0	0

* Phytotoxins were extracted from *S. scabies* grown on six different media and on potato slices, and applied to the mini-tubers. Acetone and an extract from uninoculated oatmeal agar were used as controls.

† Twenty tubers were used for each treatment and scored for severity of lesion formation as follows: —, healthy tuber; +, dark brown skin or lenticles; ++, blackened tissue; +++, blackened and erupted skin.

the lesions induced by pathogen infection (Fig. 1.) The scab symptoms included browning and eruption of the tuber's skin at the site of phytotoxin application. These symptoms were apparent about 24 h after phytotoxin application and increased in severity over time. The lesions induced by the *S. scabies* spores appeared to be somewhat more severe than the lesions resulting from phytotoxin application. This difference may be due to continual production of phytotoxins by the pathogen at the site of infection.

Control experiments were done to ensure that lesions induced by phytotoxins were due to the activity of the phytotoxin and not to contamination of the soil, tuber or phytotoxin with the pathogen. The scab lesions induced by phytotoxin application were examined for the presence of the pathogen. Sections of the tuber containing a lesion were subcultured on 5% (w/v) water agar medium (Harrison, 1962). *S. scabies* was not recovered from the lesion using this method. Phytotoxin preparations were also inoculated onto oatmeal agar plates, and no evidence of spore contamination was apparent. These results indicate that the scab lesions induced by phytotoxin application were due to phytotoxin activity and not to contamination with *S. scabies*.

To determine the number of compounds produced that had scab-inducing activity, the components of a phytotoxin extract from *S. scabies* grown on oatmeal agar medium were separated by silica gel TLC using chloroform/methanol (9:1). The silica gel TLC plate was fractionated and the compounds were eluted. These samples were dried and suspended in acetone and applied to Kennebec mini-tubers to assay for pathogenicity.

Fig. 1. Scab lesions on Pontiac mini-tubers. Pontiac mini-tubers were treated with (a) acetone, (b) *S. scabies* RB2 spores, or (c) a phytotoxin extract from *S. scabies* RB2 grown in oatmeal broth medium. The darkened areas on the tubers in (b) and (c) correspond to scab lesions.

Only fractions containing yellow compounds possessed scab-inducing activity (data not shown). This is consistent with the yellow-pigmented thaxtomins compounds isolated by Lawrence *et al.* (1990) from *S. scabies* grown on sterilized potato slices and suggested that the phytotoxins produced by *S. scabies* on oatmeal medium might be similar to the previously characterized thaxtomins.

Phytotoxin production during growth in oatmeal broth medium

Production of phytotoxins by *S. scabies* was studied during growth of the organism in oatmeal broth medium. In initial experiments, both the cell pellet and cell filtrate were extracted and tested for the presence of lesion-inducing compounds. Only the culture filtrate contained biological activity, indicating that the phytotoxin was either loosely associated with the mycelial surface or was secreted into the growth medium. As with the phytotoxin preparations from cells grown on oatmeal agar medium

Fig. 2. Time course of phytotoxin production in oatmeal broth medium. *S. scabies* RB2 was grown for 72 h in oatmeal broth. Growth was measured as OD₆₅₀ (a). Phytotoxins were isolated from the cell filtrate at various times. Equivalent amounts of extract were analysed by silica TLC (b). Five yellow-pigmented compounds were observed and labelled compounds 1–5.

Table 2. Characteristics of thaxtomin compounds produced by *S. scabies* in oatmeal broth

Compound	Silica gel R_F *	Molecular mass†
1	0.31	438
2	0.33	438
3	0.40	392
4	0.46	422
5	0.50	406

* Extracts were separated by silica gel TLC in chloroform/methanol (9:1).

† Determined by FAB mass spectroscopy.

and potato slices (Table 1), the extract from the oatmeal broth growth medium was yellow.

To monitor phytotoxin production during growth, culture filtrate samples were taken at various times during the growth cycle for compound extraction. Equivalent volumes of each sample were analysed by silica gel TLC (Fig. 2). No yellow-pigmented compounds were detected in culture filtrates of exponentially growing cells. However, when the culture reached late exponential to early stationary phases of growth (24 to 33 h), five yellow compounds were visible on the TLC plate.

The five yellow compounds had similar R_F values on silica gel TLC to five thaxtomins which were recently identified from a pathogenic strain of *S. scabies* grown on sterile potato slices (Table 2) (King *et al.*, 1992). Two of the yellow-pigmented compounds with R_F values of 0.46 and 0.50 (compounds 4 and 5), were the first compounds to be detected, after 24 h culture growth. Compounds 1 and 2 were not visible at 24 h growth, but appeared in the 27 h culture and increased in intensity during the stationary phase. Compound 3 also appeared at 27 h, and was visible throughout the stationary phase. The appearance of compounds 4 and 5 from only 24 to 36 h growth suggests that these compounds may be precursors in the synthesis of compounds 1 and 2.

Chemical identification of thaxtomins

To establish the structural identity of the five yellow compounds produced by *S. scabies* grown in oatmeal broth medium, the compounds were purified by silica gel TLC, reverse-phase C₁₈ TLC and reverse-phase C₁₈ HPLC. The molecular masses of the five compounds were determined by FAB mass spectroscopy (Table 2). Electron impact mass spectroscopy and proton NMR analysis confirmed that the structures of these com-

pounds were identical to the group of five thaxtomins (compounds 1–5) recently reported by King *et al.* (1992). The results indicate that *S. scabiei* does not need to grow on the potato tuber *per se* in order to produce thaxtomins. Compound 1 (thaxtomin A) was the major product produced by *S. scabiei* RB2 when grown on oatmeal (about 4 mg (g cell dry wt)⁻¹, Fig. 2); this was the major compound produced by *S. scabiei* grown on potato slices (King *et al.*, 1992; this study). King *et al.* (1992) reported that very little compound 3 was produced by *S. scabiei* on potato slices, but sufficient amounts were produced by *S. scabiei* RB2 in oatmeal broth medium for isolation and characterization.

Regulation of thaxtomin biosynthesis by S. scabiei grown in oatmeal broth medium

S. scabiei RB2 was grown in oatmeal broth medium at 25, 28, 30 and 32 °C to optimize conditions for phytotoxin production. The strain did not grow well at temperatures below 25 °C or above 32 °C. The cultures of *S. scabiei* were harvested 24 h after the cells had gone into stationary phase at each temperature. Cell filtrates were extracted, and the amount of thaxtomin A produced (g cell dry wt)⁻¹ was measured. The optimal temperature for production of thaxtomins was determined to be 28 °C (data not shown). Production of thaxtomin A by *S. scabiei* at 28 °C was 26% higher than production at 25 °C, 11% higher than production at 30 °C, and 37% higher than when cells were grown at 32 °C.

Among streptomycetes there are many examples of secondary metabolites whose production is repressed by glucose (Demain, 1989). Since glucose was a component of media which did not support thaxtomin production by *S. scabiei*, the effects of glucose on production were studied. Cultures of *S. scabiei* RB2 were grown in oatmeal broth supplemented with 0, 0.1, 0.2 and 0.5% glucose (w/v) for 72 h at 28 °C, and the amount of thaxtomin A in each extract was quantified by HPLC. With 0.5% glucose, there was a 130-fold reduction in thaxtomin production compared to the control culture grown without the addition of glucose (data not shown). Even the addition of 0.1% and 0.2% glucose to oatmeal broth medium reduced phytotoxin production by 13% and 38%, respectively. The repressing levels of glucose in the agar media tested previously (Table 1) may explain the lack of phytotoxin production by *S. scabiei* when grown under these conditions.

The structure of the thaxtomins led us to speculate that the biosynthesis of these compounds may require aromatic amino acids as substrates or that biosynthesis may depend on early steps in the aromatic amino acid biosynthetic pathway. To address this possibility,

Table 3. Effect of amino acid addition on thaxtomin production

Addition*	Thaxtomin A production†
None	1.0
Alanine	1.0
Tryptophan	0.11
Tyrosine	0.28

* Duplicate cultures of *S. scabiei* RB2 were grown in oatmeal broth, either unsupplemented or supplemented with the indicated amino acid at 2.5 mM, for 72 h at 28 °C.

† The amount of thaxtomin A in each extract was determined by HPLC, corrected for culture dry wt, and expressed relative to production in the control culture.

2.5 mM-tyrosine or tryptophan were added to the oatmeal broth medium to assess their effect on thaxtomin biosynthesis. These amino acids were found to significantly inhibit biosynthesis of thaxtomin A (Table 3). The addition of 2.5 mM-alanine, however, did not affect thaxtomin synthesis, indicating that inhibition was not a general effect of amino acid addition to the culture. When 2.5 mM-ammonium hydroxide was added to the oatmeal broth, thaxtomin production was not inhibited (data not shown), indicating that the inhibition caused by tyrosine and tryptophan was not due to the increased nitrogen levels but rather to a specific effect of these two amino acids on thaxtomin production. When the amino acids or ammonia concentrations in the oatmeal broth were increased to 10 mM or above, thaxtomin synthesis was strongly inhibited (data not shown). These results indicate a negative effect of nitrogen on thaxtomin production, consistent with the observed inhibitory effects of nitrogen on the production of some secondary metabolites (Shapiro, 1989).

Discussion

Thaxtomins are phytotoxins produced by *S. scabiei* that may play a central role in inducing scab lesion formation on the tuber. A study by King *et al.* (1991) has shown that all pathogenic strains of *S. scabiei* tested produced thaxtomins. This correlation of thaxtomin production with pathogenicity has prompted us to study the regulation of thaxtomin biosynthesis. Our studies have shown that growth of the pathogen on potato is not required for production of phytotoxin by *S. scabiei*. Oatmeal agar medium supported production of phytotoxins by the pathogen, whereas no production of phytotoxin could be detected when the organism was grown on the other media tested. It is interesting to note that the nutritional composition of potato and oatmeal are very similar (USDA, 1982; USDA, 1984). There may

be common components of oatmeal and potato which support production of phytotoxins by *S. scabies*.

We have demonstrated that all five of the reported thaxtomins (King *et al.*, 1992) were secreted by *S. scabies* grown on oatmeal broth medium. The chemical structures of these compounds were confirmed by FAB mass spectroscopy and proton NMR. The production of these phytotoxins during the late exponential and early stationary phases of growth is consistent with the expression of secondary metabolites by other species of *Streptomyces* as well as the production of phytotoxins by certain other bacteria. For example, syringomycin, produced by *Pseudomonas syringae* pv. *syringae*, is a phytotoxin associated with numerous diseases of dicots and monocots. This phytotoxin is produced in synthetic medium by the bacteria as the culture reaches the stationary phase of growth (Gross, 1985), similar to phytotoxin production by *S. scabies*. Not all phytotoxic secondary metabolites, however, are produced solely during the stationary phase of growth. Phaseolotoxin, a phytotoxin associated with halo-blight of bean, is produced constitutively during growth by *Pseudomonas syringae* pv. *phaseolicola* (Nuske & Fritsche, 1989).

The same five phytotoxins are produced by *S. scabies* when grown on either potato slices (King *et al.*, 1992) or in oatmeal broth (this study). There are several advantages, however, to studying thaxtomin biosynthesis in the liquid oatmeal broth medium compared to studying thaxtomin production when *S. scabies* is grown on potato. The oatmeal medium is easier to prepare, and the bacteria grow faster in this medium than on potato slices. The isolation and purification of thaxtomins from cell filtrates is much easier, since there are fewer compounds co-isolated with thaxtomins in initial extractions. The timing of appearance of the five compounds relative to each other during the different growth stages of the culture can be monitored to suggest potential intermediates in the thaxtomin biosynthetic pathway. Another advantage to studying biosynthesis of these compounds on a synthetic medium is the opportunity to study the regulation of biosynthesis.

Differential production of these five compounds during growth (Fig. 2) of *S. scabies* led us to propose a pathway for the latter stages of thaxtomin biosynthesis (Fig. 3). Compound 3 is the logical precursor for the synthesis of the other four compounds since it is the least modified of the compounds and was detected early in stationary phase. Compounds 4 and 5 were also detected early in stationary phase, but the levels of these compounds quickly declined when compounds 1 and 2 were detected. Compound 5 would be the product of an N-methylation in the dioxopiperazine ring of compound 3. Compound 5 is converted to compound 4 by a hydroxylation at the C-14 position. Unlike compounds 4

Fig. 3. Proposed pathway for the later stages of thaxtomin biosynthesis. The relative amounts of compounds 1 and 2 produced in the later stages of growth were determined by comparing the areas of their HPLC elution peaks.

and 5, we have consistently detected low level amounts of compound 3 throughout stationary phase. The presence of low levels of compound 3 may be due to cleavage of the N-methyl and hydroxyl groups from the other compounds at a low frequency or to the inability of the enzyme involved in the methylation of compound 3 to convert all of the substrate to compound 5.

As the levels of compounds 4 and 5 declined, compounds 1 and 2 were accumulated. These steps involve the hydroxylation of compound 4 at the C-19 position (compound 1) or at the C-20 position (compound 2). Compound 1 (thaxtomin A) was the major product detected under all growth conditions tested, similar to what we found when *S. scabies* RB2 was grown on potato slices. The hydroxylation of compound 4 may either involve two enzymes which are specific for hydroxylation at the ortho or meta positions or one enzyme which is not as specific.

Thaxtomin production in oatmeal broth medium was subject to the glucose repression which has been observed for many of the secondary metabolites produced by streptomycetes (Demain, 1989). Since levels as low as 0.1% glucose will repress production of these compounds, any medium with glucose as its carbon source

would likely not support production. These results may explain why phytotoxin production by *S. scabies* grown on synthetic media was not detected in previous studies (Lawrence *et al.*, 1990) or in the other growth media tested in this study (Table 1).

Repression of phytotoxin production by glucose is consistent with the *S. scabies* micro-environment. *S. scabies* infects the potato through newly formed lenticels or wounds, but does not invade through intact skin (Davis & Garner, 1978; Adams, 1975). *S. scabies* infection occurs early in tuber development, during which time the level of glucose in the potato peel is less than 0.1% (Goto, 1981). This non-repressive level of glucose would allow phytotoxin production by *S. scabies* during the early stages of infection. Subsequent damage to the potato tissue by the phytotoxin may increase ability of the pathogen to invade the potato and may provide nutrients for bacterial growth. In streptomycetes, the mechanism of glucose repression of secondary metabolite production is not well understood, and apparently does not involve cAMP (Demain, 1989).

The structure of thaxtomin is similar to the aromatic amino acids. We have demonstrated that thaxtomin biosynthesis was inhibited by the addition of tryptophan or tyrosine to the oatmeal broth medium. If these amino acids were simply substrates for biosynthesis we might expect that their addition would increase the production of thaxtomin. However, thaxtomin biosynthesis was inhibited by millimolar tyrosine or tryptophan. The 2.5 mM concentrations of these amino acids inhibiting thaxtomin biosynthesis are comparable to those which inhibit synthesis of 3-deoxy-D-arabino-heptulosonate-7-phosphate synthase in *Escherichia coli* cultures (Brown & Doy, 1963; Smith *et al.*, 1962). This enzyme carries out the initial step of the shikimate pathway leading to aromatic amino acid biosynthesis. However, it has been proposed that unlike *E. coli* and other enterics, aromatic amino acid biosynthesis in the streptomycetes is regulated by growth rate rather than feedback repression (Hood *et al.*, 1992). If aromatic amino acid biosynthesis in *S. scabies* is not regulated by endproduct repression, then inhibition of thaxtomin biosynthesis by these aromatic amino acids must occur through another route. One possibility is that the aromatic amino acid precursors required for thaxtomin production are synthesized by a dedicated pathway regulated by feedback inhibition. Alternatively, a branch of the aromatic amino acid pathway which is specific for thaxtomin production may itself be feedback-regulated by aromatic amino acids.

In this study we have demonstrated that milligram quantities of thaxtomin A can be readily produced and purified. The ability to produce these compounds in large quantities provides the means for studying thaxtomin A biosynthesis and for potentially using thaxtomin A

application as a screen to test potato cultivars for resistance to scab disease.

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