Differential Production of Thaxtomins by Pathogenic *Streptomyces* Species In Vitro

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This research was funded by the Binational Research and Development Project No. US-2087-91F.
We thank E. Nelson for assistance with HPLC analyses.
Accepted for publication 1 February 1995.

ABSTRACT

Loria, R., Bukhalid, R. A., Creath, R. A., Leiner, R. H., Olivier, M., and Steffens, J. C. 1995. Differential production of thaxtomins by pathogenic *Streptomyces* species in vitro. Phytopathology 85:537-541.

Streptomyces scabies and *S. acidiscabies*, causal agents of potato scab, produced the phytotoxin thaxtomin A and, to a lesser extent, other thaxtomins in oatmeal broth (OMB). Two nonpathogenic strains, *Streptomyces lividans* TK 24 and *Streptomyces* sp. 84-05, did not produce thaxtomins in OMB. Though thaxtomin A was produced by *S. scabies* strain 87-22 in potato broth, fresh potato starch, and some commercial starch preparations, production was much greater in OMB than in other media tested. Thaxtomin A was not produced in Luria broth or tryptic soy broth. Production of thaxtomin A by 87-22 was suppressed by 0.5% glucose in OMB but was stimulated by up to 5.0% glucose in oatmeal agar. *Streptomyces scabies* strain 87-22 produced 4.25 µg thaxtomin A per milliliter of OMB while strain 84-34 produced 0.17 µg per milliliter of OMB. *Streptomyces acidiscabies* strains 84-110 and 90-25 produced similar amounts of thaxtomin A, 2.65 and 4.45 µg per milliliter of OMB, respectively. Strain 87-22 was much more virulent on tubers of Chippewa, a scab-susceptible potato cultivar, than was strain 84-34. A tuber slice bioassay was useful for detection of thaxtomins in culture media and for identifying pathogenic *Streptomyces* strains.

Additional keywords: acid scab, common scab, streptomycetes, toxin.

Streptomyces species are filamentous prokaryotes that have well-differentiated sporulating mycelium. These organisms produce a multitude of secondary metabolites with biological activity, including many antibiotics. The chemistry, biosynthesis, molecular genetics, and regulation of antibiotic production have been studied extensively (3,9,26) because of their commercial importance. Antibiotics presumably provide streptomycetes with a competitive advantage in soil, where most species exist as saprophytes. However, the biological activity of secondary metabolites produced by *Streptomyces* species is not limited to antimicrobial properties. At least one secondary metabolite, bialaphos, produced by *Streptomyces hygroscopicus* and *S. viridochromogenes*, has herbicidal properties. The biosynthetic genes for this compound have been cloned and characterized (22).

Genetic regulation of antibiotic biosynthesis is quite complex (3,4,6). Production of secondary metabolites, including antibiotics, by *Streptomyces* is often associated with a low growth rate (7). Concentrations of secondary metabolites are usually induced in culture media at the end of the exponential growth phase, at the same time that aerial mycelium is produced in surface cultures. Catabolic repression of secondary metabolism is also well documented; both the type and concentration of carbon and nitrogen sources greatly affect antibiotic production (23).

Streptomyces scabies (16) a causal agent of potato (*Solanum tuberosum* L.) scab, produces five related phytotoxins, called thaxtomins, which are unique 4-nitroindol-3-yl-containing 2,5 dioxopiperazines (12,14). These toxins, which were isolated from naturally infected tubers and tuber slices colonized with the pathogen, produce necrosis on immature potato tubers in vitro (18). Production of thaxtomins in potato tuber tissue was correlated with plant pathogenicity among 28 *S. scabies* and several saprophytic *Streptomyces* strains (13). Thaxtomin A is the dominant toxin produced by *S. scabies* in colonized host tissue and exceeds thaxtomin B, the next most abundant thaxtomin, by a ratio of approximately 20:1 (13). *Streptomyces acidiscabies* (17), the cause of acid scab (21), and *S. ipomoea*, the cause of sweet potato soil rot and pox (24), also produce thaxtomins in plant tissue (12).

We hypothesized that thaxtomin A could be produced by *S. scabies* and *S. acidiscabies* in culture media, as well as tuber tissue, and that the regulation of thaxtomin biosynthesis is similar to that of other secondary metabolites produced by streptomycetes. This report describes the production of thaxtomin A in culture media by strains of *S. scabies* and *S. acidiscabies*, but not by two nonpathogenic *Streptomyces* species, and suppression of thaxtomin A production by glucose. Differential virulence of two strains of *S. scabies* that differ in the quantity of thaxtomin A produced also was demonstrated. A potato tuber slice assay suitable for evaluation of pathogenicity of *Streptomyces* spp. and thaxtomin production is described.

MATERIALS AND METHODS

Strains and media. The pathogenic strains used in this study were *S. scabies* 84-34 and 87-22, and *S. acidiscabies* 84-110 and 90-25. The nonpathogenic strains *Streptomyces lividans* TK24 and *Streptomyces* species 84-05 were included as controls. All strains except *S. lividans* TK24 were isolated from potato scab lesions by R. Loria and pathogenicity was confirmed in greenhouse studies using the methods described by Loria and Davis (19). Strains were stored as spore suspensions in glycerol (20%) at –20 and –80 C, as described by Hopwood et al (11) and cultured on ISP 2 (Difco Laboratories, Detroit MI) at 30 C for 7–10 days to produce spores. Washed spores (11) were used in all experiments in which nutritional factors were evaluated.

Thaxtomin A production was evaluated in several media. Oatmeal broth (OMB) was prepared by boiling rolled oats (20 g/l) in distilled water for 20 min and straining through cheesecloth. Oatmeal broth and OMB agar (1.5%, OMA) were prepared with 0.5, 2.0, and 5.0% glucose in some experiments. Several potato

and starch media were used. Potato broth (PB) was prepared by cooking peeled potato tubers (200 g/l) and straining through cheesecloth, then diluting in water to 0.5, 1.0, 2.0, 5.0, 10.0, and 20.0%, based on tuber fresh weight. A crude fresh potato starch preparation was prepared from potato tubers by homogenizing peeled tubers in a blender and washing repeatedly by centrifugation to remove soluble components. The crude starch (0.19, 0.38, and 1.9% dry weight) was reconstituted in basal salts solution (11) without glucose, substituting $(NH_4)_2SO_4$ for asparagine. Crude fresh starch was compared with purified starch from potato (Sigma Chemical Co., St. Louis, MO), amylopectin from potato (Sigma) and soluble starch from potato (Difco) all at 1.75% dry weight in basal salts. Luria Broth (LB, Sigma) and tryptone soy broth (TSB, Sigma) were also used. All media were adjusted to pH 7.2–7.4 and dispensed in 200-ml aliquots in 500-ml Erlenmeyer flasks before autoclaving. In some experiments, agar (1.5%) was added to media. Trace elements (11) were added after autoclaving.

Concentrations of glucose, fructose, and sucrose in PB and OMB media were determined by the UV method (2) using a kit from Boehringer Mannheim (Indianapolis, IN) according to the manufacturer's instructions.

Liquid media were inoculated with spore suspensions (approximately 10^6 spores per flask) and incubated on a rotary shaker at 150–180 rpm and 28 C for 3–4 days. The cultures were filtered through two layers of miracloth before extraction and the dry weight of mycelium was determined. Solid media were streaked with spore suspensions and incubated at 28 C for 7–10 days. Relative growth of strains on solid media was estimated as described by Shirling and Gottlieb (27). All experiments were repeated at least once with similar results.

Extraction and chromatography of thaxtomin A. Authentic thaxtomin A (14) was graciously provided by R. R. King. Methods for extraction, purification, and identification of thaxtomins were modifications of those described by King et al (12). Filtered liquid cultures (200 ml) were extracted four times with chloroform (100 ml). Agar cultures were homogenized in a blender with acetone, filtered, and washed twice with acetone (100 ml) . The acetone was then evaporated and the aqueous residue was extracted with chloroform. The crude extract that remained when the chloroform was evaporated to dryness was stored in the dark at 4 C until used for bioassays or chromatography. Crude extracts were dissolved in 1 ml of chloroform and loaded onto Whatman LKC-18F reversed phase silica thin layer chromatography (TLC) plates, and run in acetone and water (3:2) in a saturating environment. The yellow bands were eluted from the silica and rechromatographed on Whatman PLK5 silica gel TLC plates and run in chloroform and methanol (9:1). Alternatively, the chloroform extract was run directly on the PLK5 silica gel plates. The yellow band comigrating with authentic thaxtomin A was again eluted and dissolved in chloroform, and evaporated to dryness. The resulting orange powder was stored in a dessicator at 4 C and used for Fast Atom Bombardment (FAB) mass spectroscopy and as a standard in high-pressure liquid chromatography (HPLC) assays.

Identification and quantification of thaxtomins. Thaxtomins were identified and quantified by HPLC using a Waters 600E Lambda-Max Model 481 Le spectrophotometer (Millipore Waters Milford, MA) with a NovaPak C18 column (4 μm particle size, 4.6 × 120 mm) and a Hewlett Packard 3393A integrator. Phytotoxins were eluted with a 25–50% acetonitrile gradient over 20 min and monitored at A_{380} (1). A standard curve for thaxtomin A was established with dilutions of a known quantity of the purified compound. Ultraviolet and visible spectroscopic analyses were performed with a Beckman UV/vis DU-65 spectrophotometer using extinction coefficients as determined by King et al (12). FAB mass spectra were acquired with a reverse geometry VG ZAB-SE mass spectrometer. Values presented for thaxtomin A production are for an individual experiment but were repeatable.

Tuber slice assays. A tuber slice assay was used to confirm the phytotoxicity of culture extracts and purified thaxtomin A. The scab-susceptible cultivar Chippewa was grown in the greenhouse from certified seed tubers. Progeny tubers (3–4 cm in diameter) were harvested immature, and stored at 4 C for at least 2 mo before they were used, to reduce nonspecific browning. Tubers were surface sterilized in 0.5% NaOCl and cores (1.5 cm diameter) of pith tissue were aseptically removed from the center of the tuber. Cores were sliced into disks (0.25 cm thick) and placed on moist sterile filter paper in petri dishes. Two to four replicate tuber disks were used per treatment. Test compounds were dissolved in methanol and applied to sterile filter paper disks (0.5 cm diameter). The disks were air dried before placing them onto the tuber slices. A drop of sterile distilled water was applied to each disk to facilitate movement of the test compounds onto the tuber surface. Tubers were incubated in a moist chamber at 22–24 C in the dark. Slices were observed for necrosis and collapse of tuber cells under and surrounding the disks.

The tuber slice assay was also used to assess the necrotizing ability of *Streptomyces* strains used in media production experiments. Cultures of test strains were grown on OMA for 5–7 days at 28 C and agar plugs from the sporulating colonies were inverted onto the tuber disks. Disks were incubated in a moist chamber at 22–24 C in the dark.

All tuber slice assays were repeated three to four times with similar results.

Virulence assays. Minitubers were produced from stem cuttings, as described by Loria and Kempter (20). Briefly, potato plants of the cultivar Chippewa were grown from tubers in the greenhouse and induced to tuberize by exposure to short days (9-h photoperiod) and cool temperatures (21 C day, 15 C night) for a minimum of 10 days. Leaf bud cuttings were taken from plants, placed in vermiculite, incubated in a mist chamber for 13 days, then transplanted into vermiculite colonized by *S. scabies*, or noninoculated vermiculite. Ten replicate tuberizing cuttings per treatment were incubated for 7 days in high humidity at 24 C with an 18-h photoperiod. Disease severity was evaluated as percent surface area infected. Infested vermiculite was prepared by growing *S. scabies* in ISP 1 broth (Difco) for 48 h at 30 C, saturating sterile vermiculite with the inoculum (150 ml of inoculum per 2.5 L of vermiculite), then incubating for 7 days at 30 C. Inoculum density was estimated by dilution plating onto ISP 2 (Difco). This study was repeated twice with similar results and analyzed with the SAS general linear model (SAS, Cary, NC).

RESULTS

Identification of thaxtomin A produced by 87-22 in OMB. A yellow tint developed in OMB cultures of 87-22 within 2–3 days at 28 C and increased in intensity until cultures were harvested after 4 days. Chloroform extracts of the media were bright yellow and produced a strong yellow band (band 1) at $R_f = 0.65$ in reversed phase on C18 TLC, which comigrated with the thaxtomin A standard. A less intense yellow band (band 2) occurred at $R_f = 0.59$ on these plates. When band 1 was eluted and run on silica TLC plates, a single intense yellow band was visible at $R_f = 0.50$ and again comigrated with the thaxtomin A standard. Band 2 separated into two faint yellow bands on silica gel plates, with R_f values of 0.66 (band 2a) and 0.57 (band 2b). Bands 2a and 2b, presumed to be thaxtomins based on color and R_f (14), were very minor components of the $CHCl_3$ extract and were not characterized further.

Band 1 exhibited absorbance maxima at 398, 343, 279, 249, and 220 nm (ethanol), consistent with the spectral properties of thaxtomin A (12). FAB mass spectrometry of band 1 provided *m/z* 439.3 (M+H) (30%), and *m/z* 421.2 (M-H_2O) (38%), also in agreement with the mass properties of thaxtomin A (14).

HPLC analysis of band 1 showed that the 380-nm absorbing

component eluted as a single peak with a leading shoulder. The presence of two components with mass identical to thaxtomin A (438) suggests that band 1 is composed of both thaxtomin A and its *o*-hydroxybenzyl regioisomer (12). For purposes of quantifying the effect of varying culture conditions on production of thaxtomin A by *Streptomyces* spp., we integrated the area under the band 1 HPLC peak (390 nm; thaxtomin A plus its *o*-hydroxybenzyl regioisomer) and deduced the concentration of thaxtomin A by comparison to standard curves generated from solutions of characterized thaxtomin A. Band 1 (thaxtomin A plus its *o*-hydroxybenzyl regioisomer) produced necrosis on potato tuber disks within 48 h after application (data not shown). The appearance of lesions produced by this preparation was identical to that produced by the authentic thaxtomin A standard (Fig. 1).

Effects of media on thaxtomin production. *Streptomyces scabies* strain 87-22 produced 4.25 µg thaxtomin A per milliliter of OMB. However, glucose amendment of OMB greatly suppressed production of thaxtomin A at concentrations of 0.5% additional glucose or above (Fig. 2). In contrast, growth of strain 87-22 increased with increasing concentrations of glucose. The dry weight of mycelial growth of strain 87-22 was 0.232, 0.396, 0.544, and 1.045 g per 200 ml in OMB and OMB amended with 0.5, 2.0, and 5.0% glucose, respectively. Surprisingly, glucose stimulated thaxtomin A production in OMA. Strain 87-22 produced 16 µg thaxtomin A per plate (20 ml) in OMA and 65.0, 52.0, and 37.5 µg thaxtomin A per plate at 0.5, 2.0, and 5.0% glucose, respectively.

Streptomyces scabies strain 87-22 also produced thaxtomin A in 5.0 and 10.0% PB, 0.5, 1.0 and 5.0% fresh potato starch broth with basal salts, and 1.75% Difco potato starch broth with basal salts. The concentration of thaxtomin A in these media, based on the intensity of bands on TLC, was much lower than in OMB or OMA and was not quantified. Thaxtomin A was not produced in other concentrations of those media or in LB, TSB, purified starch from potato, amylopectin from potato, or soluble starch from potato.

Growth appeared to be poor in liquid media prepared from crude or purified starches, but mycelium could not be separated from the medium by filtration because of the high viscosity. To determine if poor aeration of the viscous media was inhibiting thaxtomin A production, starches were evaluated in solid media. Production of thaxtomin A in solid starch media was consistent with that obtained in liquid cultures, but growth was poor compared with OMA or ISP2.

Extracts of PB (10%) and fresh potato starch (10 and 15%) also produced a yellow band that comigrated with band 2 from OMB, on C_{18} TLC.

The sugar content of OMB and PB (10%) was 0.17 and 0.355 g/L sucrose, 0.005 and 0.144 g/L glucose, and 8.6 and 0.284 g/L fructose, respectively.

Production of thaxtomin by *S. scabies* and *S. acidiscabies* strains. Both strains of *S. scabies* (84-34 and 87-22) and *S. acidiscabies* (84-110 and 90-25) produced thaxtomin A, and small amounts of other thaxtomins, in OMB (Fig. 3). Strain 87-22 produced 4.25 µg thaxtomin A per milliliter of OMB while 84-34 produced 0.17 µg thaxtomin A per milliliter of OMB. *Streptomyces acidiscabies* strains 84-110 and 90-25 produced similar amounts of thaxtomin A, 2.65 and 4.45 µg per milliliter of OMB, respectively. Two nonpathogenic strains, *S. lividans* TK24 and *Streptomyces* sp. 84-05, did not produce thaxtomins in OMB. Growth of all strains in OMB was comparable, ranging from 0.11 to 0.27 g dry weight per 200 ml; growth was not correlated to thaxtomin A production among the producing strains.

Tuber slice assays. Purified thaxtomin A produced a necrotic reaction on tuber slices. Necrosis was first visible 24 h after application and increased in severity over several days. Severely necrotic tissue collapsed during the course of the assay, leaving a slightly sunken area on the tuber slice surface. Crude extracts of OMB cultures of those *Streptomyces* strains known to produce thaxtomin A also produced necrosis on the surface of tuber slices. The pattern and type of necrosis was identical to that produced by purified thaxtomin A. Necrosis was not observed when crude extracts of OMB cultures of nonpathogenic strains were applied to tuber slices (Fig. 1).

Strains that produced thaxtomin A in OMB also produced necrosis when inoculated onto tuber slices. Necrosis was visible beneath the OMA plug within 24 h of inoculation. Within 48 h of inoculation, a necrotic halo was apparent around the OMA plug (Fig. 4). Pathogenic strains colonized the tuber slices, causing the inoculum plugs to adhere to the surface. Nonpathogenic strains did not produce necrosis on the tuber slices or colonize the tuber surface. Inoculum plugs from 5- to 7 day-old OMA cultures produced more consistent results than from older or younger cultures, or from cultures grown on other media (data not shown).

Virulence of *S. scabies* strains. *Streptomyces scabies* strain 87-22 produced more severe scab symptoms on the scab-susceptible potato cultivar Chippewa than did strain 84-34. Strain 84-34 produced lesions primarily at the lenticels, and necrosis was superficial, extending less than 1 mm below the tuber

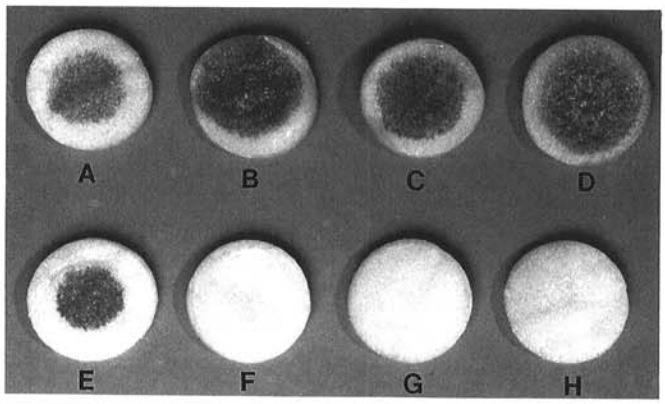

Fig. 1. Necrosis on potato tuber slices produced by thaxtomin A and crude extracts of oatmeal broth cultures of *Streptomyces* strains. **A** and **B,** *Streptomyces scabies* strains 84-34 and 87-22; **C** and **D,** *Streptomyces acidiscabies* strains 84-110 and 90-25; **E,** authentic thaxtomin A (1µg); **F,** a methanol control; and **G** and **H,** the nonpathogens *Streptomyces lividans* TK24 and *Streptomyces* sp. 84-05. Samples (10 µl) were applied to filter paper disks in methanol and air dried before placing each disk onto the tuber slices.

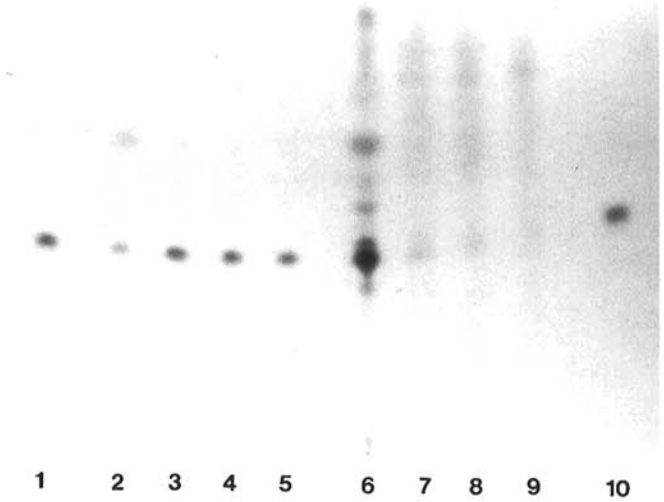

Fig. 2. Thin layer chromatograph (silica gel) of thaxtomins produced by *Streptomyces scabies* strain 87-22 in oatmeal broth (OMB) and oatmeal broth agar (OMA). Lanes 1 and 10 are authentic thaxtomin A. Lanes 2–5 are OMA amended with 0, 0.5, 2.0, and 5.0% glucose. Lanes 6–9 are OMB amended with 0, 0.5, 2.0, and 5.0% glucose.

periderm (Fig. 5). Lesions produced by strain 87-22 were not limited to the lenticels and were up to 5 mm deep. The necrotic surface area of tubers inoculated with 87-22 (60%) was significantly ($P < 0.05$) greater than that of tubers inoculated with strain 84-34 (35%) and the results from both of these treatments were significantly different from those with noninoculated tubers (5%).

DISCUSSION

Streptomyces scabies and *S. acidiscabies* produced thaxtomin A in several culture media, demonstrating that toxin production does not require induction by living host tissues or enzymatic modification by the host. Thaxtomin A was not produced by the two nonpathogenic *Streptomyces* strains tested. These data are consistent with results obtained by King et al (13) when potato tuber slices were used as a substrate for thaxtomin production. All pathogenic strains produced small quantities of other thaxtomins in OMB, based on TLC analyses. These thaxtomins have been described previously (12) and a biosynthetic pathway for the five described thaxtomins has recently been proposed (1).

The *S. scabies* strains 87-22 and 84-34 differed greatly in the quantity of thaxtomin A produced in media. The low-producing strain, 84-34, was originally isolated from an erumpent scab lesion on a field-grown tuber of Chippewa, a scab-susceptible cultivar. The high-producing strain, 87-22, was isolated from a deep-pitted lesion on Russet Burbank, a scab-resistant cultivar (R. Loria, *unpublished data*). Virulence assays demonstrated a clear difference between the two strains: 87-22 produced much more extensive and severe symptoms on potato tubers than did 84-34. These data suggest that thaxtomin A production may be correlated with virulence in *S. scabies*. Quantitative analyses of thaxtomin A production by, and virulence of, a larger collection of pathogenic *S. scabies* strains is needed to evaluate this hypothesis.

Antibiotic production in *Streptomyces* is also known to be strain specific. Strains in the same species may produce different antibiotics or different quantities of the same antibiotic, and strains in different *Streptomyces* species may produce the same antibiotics. Generally, strains that produce the same antibiotics contain the same gene clusters for antibiotic biosynthesis (23). It is likely that plant-pathogenic *Streptomyces* species have the same or similar biosynthetic gene clusters for thaxtomin production.

Though thaxtomin A was produced by strain 87-22 in several media, the best culture medium for production was unamended OMB. Growth of strain 87-22 at the higher concentrations of PB was similar to that in OMB, but the highest concentration of PB (20%) did not support thaxtomin production. Unamended PB contained much more glucose than did OMB, suggesting that the concentration of glucose in PB was sufficient to suppress thaxtomin production. When OMB was amended with glucose, thax-

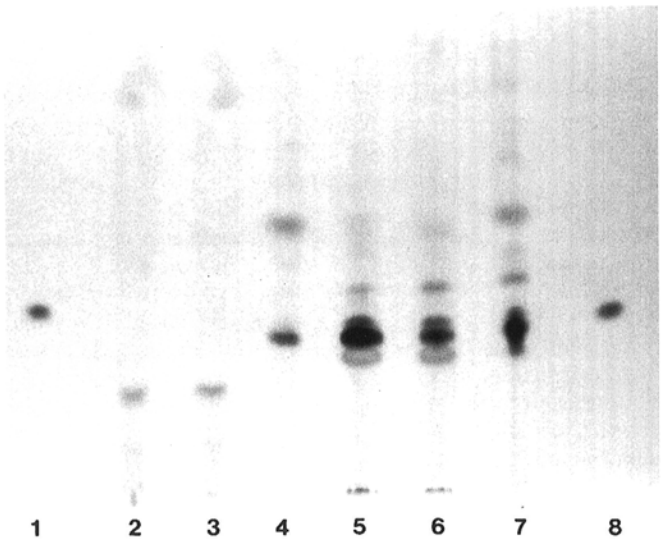

Fig. 3. Thin layer chromatograph (silica gel) of thaxtomins produced by *Streptomyces* spp. Lanes 1 and 8 are authentic thaxtomin A. Lanes 2 and 3 are the nonpathogenic strains *Streptomyces lividans* TK24 and *Streptomyces* sp. 84-05. Lanes 4 and 7 are the *Streptomyces scabies* strains 84-34 and 87-22. Lanes 5 and 6 are the *Streptomyces acidiscabies* strains 84-110 and 90-25.

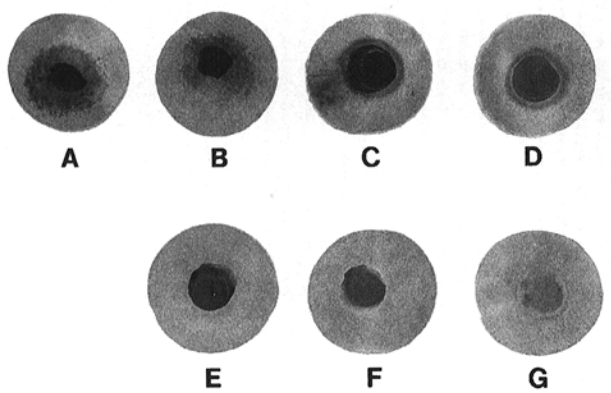

Fig. 4. Tuber slice pathogenicity test. Necrotic halos are produced around agar plugs taken from 5-day-old oatmeal agar cultures of pathogenic, but not nonpathogenic, *Streptomyces* strains. **A** and **B,** cultures of *Streptomyces scabies* strains 84-34 and 87-22; **C** and **D,** *Streptomyces acidiscabies* strains 90-25 and 84-110; **E** and **F,** the nonpathogens *Streptomyces lividans* TK24 and *Streptomyces* sp. 84-05; and **G,** a sterile oatmeal agar plug.

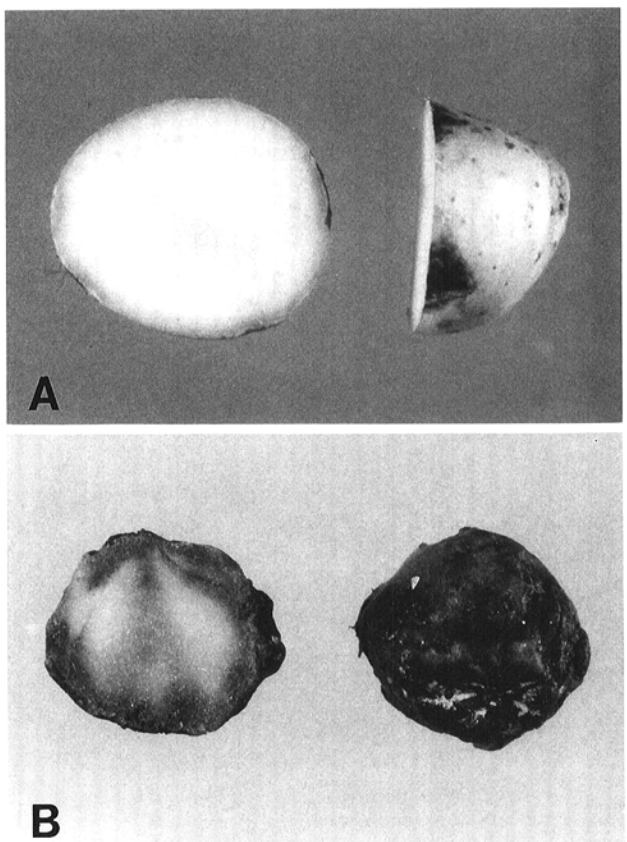

Fig. 5. Lesions produced on potato tubers by **A,** *Streptomyces scabies* strains 84-34 and **B,** 87-22, showing external and internal symptoms. Tubers were produced on stem cuttings that were inoculated 13 days after removing them from stock plants.

tomin A biosynthesis was suppressed. Another *S. scabies* strain was recently reported to produce thaxtomin A in OMB, and production was suppressed by glucose in this strain (1). Suppression of antibiotic biosynthesis by glucose is also common among *Streptomyces* species (23). The lack of suppression of thaxtomin A production by glucose at concentrations of up to 5% in OMB agar may be due to the more limited availability of this sugar in the agar matrix than in agitated liquid cultures.

Thaxtomin A production in OMB was much greater than in the other starch-based media, which are also low in glucose. However, growth in these media was relatively poor. Starch alone does not appear to be sufficient for vigorous growth of this strain. It is possible that the high concentration of fructose in OMB may stimulate growth and thaxtomin A production. Fructose enhances the production of syringomycin biosynthesis by *Pseudomonas syringae* pv. *syringae* (25).

Thaxtomin A was also not produced in LB and TSB, though growth of *S. scabies* was good. Although glucose is not a component of these (rich) media, they contain high levels of nitrogen. Suppression of thaxtomin A production in another *S. scabies* strain by tryptophan (2.5 mM), tyrosine (2.5 mM), and ammonia (10 mM) has recently been reported (1). Suppression of secondary metabolites by nitrogen in *Streptomyces* species is well known (26). Alternatively, the lack of a chemical stimulant, such as fructose, may be responsible for the absence of thaxtomin production in these media.

The tuber slice bioassay appears to be a convenient and efficient technique for assessing thaxtomin production by, and pathogenicity of, *Streptomyces* strains. Current methods for evaluating pathogenicity rely on whole tubers produced from seed pieces (19) or from stem cuttings (20). The phytotoxicity of thaxtomins has been assessed using in vitro–produced tubers (18). The tuber slice bioassay is more efficient than either of these methods, though it is not sensitive enough to provide data on the relative virulence of strains.

Streptomyces species are highly successful soil saprophytes. Their success is partly due to their ability to produce a variety of biologically active secondary metabolites, many of which inhibit other microorganisms. Relatively few *Streptomyces* species are plant pathogens. Pathogenic species are morphologically, physiologically, and genetically diverse (8,10,15,28), suggesting that plant pathogenicity in this genus arose several times or that genes required for pathogenicity have been transferred among species. Horizontal transfer of chromosomal DNA between bacteria can be carried out by plasmid-mediated conjugation, transduction, or naturally occurring transformation with fragments of chromosomal DNA (5). Gene exchange in nature among *Streptomyces* strains has been reported (6).

Streptomyces scabies, *S. acidiscabies,* and *S. ipomoea* are all known to produce thaxtomins (12), suggesting that this family of phytotoxins may be a pathogenicity determinant in *Streptomyces* species. Thaxtomin biosynthetic genes may have been transferred among soil-inhabiting *Streptomyces* species living in close association with potato tubers and other hosts. The quantity of the individual thaxtomins produced may differ among strains and species and could explain differences in virulence. Thaxtomin A appears to be the dominant toxic compound produced by *S. scabies* and *S. acidiscabies*, since shifting strains and media did not result in an increase in other thaxtomins. The molecular basis for quantitative differences in ability to biosynthesize and accumulate thaxtomins remains uncertain.

LITERATURE CITED

1. Babcock, M. J., Eckwall, E. C., and Schottel, J. L. 1993. Production and regulation of potato-scab-inducing phytotoxins by *Streptomyces scabies*. J. Gen. Microbiol. 139:1579-1586.
2. Bergmeyer, H. U., and Bernt, E. 1974. Glucose. Pages 1176-1179 in: Methods of Enzymatic Analysis. H. U. Bergmeyer, ed. Academic Press, London.
3. Chadwick, D. J., and Whelan, J., eds. 1992. Secondary Metabolites: Their Function and Evolution. John Wiley & Sons, Chichester, England.
4. Chater, K. C. 1992. Genetic regulation of secondary metabolites in *Streptomyces*. Pages 144-156 in: Secondary Metabolites: Their Function and Evolution. D. J. Chadwick and J. Whelan, eds. John Wiley & Sons, Chichester, England
5. Chater, K. F., Henderson, D. J., Bibb, M. J., and Hopwood, D. A. 1988. Genome flux in *Streptomyces coelicolor* and other streptomycetes and its possible relevance to the evolution of mobile antibiotic resistance determinants. Pages 7-49 in: Transposition. A. J. Kingsman, K. F. Chater, and S. M. Kingsman, eds. Cambridge University Press, Cambridge.
6. Chater, K. F., and Hopwood, D. A. 1989. Diversity of bacterial genetics. Pages 23-52 in: Genetics of Bacterial Diversity. D. A. Hopwood and K. F. Chater, eds. Academic Press, London.
7. Demain, A. L., Aharonowitz, Y., and Martin, J. F. 1983. Metabolic control of secondary biosynthetic pathways. Pages 49-72 in: Biochemistry and Genetic Regulation of Commercially Important Antibiotics. L. C. Vining, ed. Addison-Wesley, Reading, MA.
8. Doering-Saad, C., Kampfer, P., Shulamit, M., Kritzman, G., Schneider, J., Zakrzewska-Czerwinska, J., Schrempf, H., and Barash, I. 1992. Diversity among *Streptomyces* strains causing potato scab. Appl. Environ. Micro. 58:3932-3940.
9. Goodfellow, M., Williams, S. T., and Mordarski, M. 1988. Actinomycetes in Biotechnology. Academic Press, London.
10. Healy, F. G., and Lambert. D. H. 1991. Relationships among *Streptomyces* spp. causing potato scab. Int. J. Syst. Bacteriol. 41:479-482.
11. Hopwood, D. A., Bibb, M. J., Chater, K. F., Kieser, T., Burton, C. J., Kieser, H. M., Lydiak, D. J., Smith, C. P., Ward, J. M., and Schrempf, H. 1985. Genetic Manipulation of *Streptomyces*: A Laboratory Manual. Academic Press, London.
12. King, R. R., Lawrence, C. H. and Calhoun, L. A. 1992. Chemistry of phytotoxins associated with *Streptomyces scabies,* the causal organism of potato scab. J. Agric. Food Chem. 40:834-837.
13. King, R. R., Lawrence, C. H., and Clark, M. C. 1991. Correlation of phytotoxin production with pathogenicity of *Streptomyces scabies* isolates from scab infected potato tubers. Am. Potato J. 68:675-680.
14. King, R. R., Lawrence, C. H., Clark, M. C., and Calhoun, L. A. 1989. Isolation and characterization of phytotoxins associated with *Streptomyces scabies*. J. Chem. Soc. Chem. Commun. 13:849-850.
15. Labeda, D. P. 1992. DNA-DNA hybridization in the systematics of *Streptomyces*. Gene 115:249-253.
16. Lambert, D. H., and Loria, R. 1989. *Streptomyces scabies* sp. nov. nom. rev. Int. J. Syst. Bacteriol. 39:387-392.
17. Lambert, D. H., and Loria, R. 1989. *Streptomyces acidiscabies* sp. nov. Int. J. Syst. Bacteriol. 39:393-396.
18. Lawrence, C. H., Clark, M. C., and King, R. R. 1990. Induction of common scab symptoms in aseptically cultured potato tubers by the vivotoxin, thaxtomin. Phytopathology 80:606-608.
19. Loria, R., and Davis, J. R. 1988. *Streptomyces scabies*. Pages 114-119 in: Laboratory Guide for Identification of Plant Pathogenic Bacteria. N. W. Schaad, ed. American Phytopathological Society, St. Paul, MN.
20. Loria, R., and Kempter, B. A. 1986. Relative resistance of potato tubers produced from stem cuttings and seed-piece-propagated plants to *Streptomyces scabies*. Plant Disease 70:1146-1148.
21. Manzer, F. E., McIntyre, G. A., and Merriam, D. C. 1977. A new potato scab problem in Maine. Univ. Maine Orono, Life Sci. Agric. Exp. Stn. Tech. Bull. 85.
22. Murakami, T., Anzai, H., Imai, S., Satoh, A., Nagaoka, K., and Thompson, C. J. 1986. The bialaphos biosynthetic genes of *Streptomyces hygroscopicus*: molecular cloning and characterization of the gene cluster. Mol. Gen. Genet. 205:42-50.
23. Okami, Y., and Hotta, K. 1988. Search and discovery of new antibiotics. Pages 33-67 in: Actinomycetes in Biotechnology. M. Goodfellow, S. T. Williams, and M. Mordarski, eds. Academic Press, London.
24. Person, L. H., and Martin, W. J. 1940. Soil rot of sweet potatoes in Louisiana. Phytopathology 30:913-926.
25. Quigley, N. B., and Gross, D. C. 1994. Syringomycin production among strains of *Pseudomonas syringae* pv. *syringae*: Conservation of the *syrB* and *syrD* genes and activation of phytotoxin production by plant signal molecules. Mol. Plant-Microbe Interact. 7:78-90.
26. Shapiro, S. 1989. Nitrogen assimilation in actinomycetes and the influence of nitrogen nutrition on actinomycete secondary metabolism. Pages 135-211 in: Regulation of Secondary Metabolism in Actinomycetes. S. Shapiro, ed. CRC Press, Boca Raton, FL.
27. Shirling, E. B., and Gottlieb, D. 1966. Methods for characterization of *Streptomyces* species. Int. J. Syst. Bacteriol. 16:313-340.
28. Tashiro, N., Miyashita, K. and Suzui, T. 1990. Taxonomic studies on the *Streptomyces* species isolated as causal agents of potato common scab. Ann. Phytopathol. Soc. Jpn. 56:73-82.