Field Isolates of *Streptomyces* Differ in Pathogenicity and Virulence on Radish

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ABSTRACT

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Common scab is a significant disease of potato, and affects root and tuber crops worldwide. Streptomycetes, a diverse group of soil-inhabiting gram-positive bacteria, cause common scab. To better understand the basis for variability in disease symptoms seen in field situations, streptomycetes were isolated from scabby potato plants. Isolates differed in morphology and pigmentation. Isolates were evaluated for pathogenicity and virulence in radish. Scab lesions varied in appearance and severity. Disease symptoms also included plant stunting, wilting, necrosis, and death. Some pathogenic isolates were missing genes from the putative pathogenicity island (PAI); several lacked the *nec1* gene, and one was missing the *txtA* gene encoding thaxtomin biosynthesis, the most reliable pathogenicity determinant. Studies of disease severity over 5 logs initial inoculum density showed that there is a threshold inoculum density for disease. Disease severity increased with inoculum density over three logs, then reached a maximum, which is characteristic of individual *Streptomyces* strains. Lesion severity was not correlated with presence of melanin, the *nec1* gene, or whether an isolate reduced seedling emergence or plant survival. Differences in disease symptoms and severity combined with absence of known pathogenicity determinants (*txtA*) or factors (*nec1*) suggest that there may be pathogenicity factors in addition to thaxtomin.

Additional keywords: phytotoxicity; *Raphanus sativus*

Common scab is an important potato disease, reducing the quality of tubers, sometimes to the point that they no longer can be sold. Common scab also affects other tuber and root crops, including radish, beet, carrot, turnip, and sweet potato, where it may produce considerable economic losses. Incidence and severity of common scab on potato are variable from year to year, field to field, and cultivar to cultivar. Reasons for this variability are not well understood, although environmental factors, including soil moisture and soil pH, and differences in cultivar susceptibility have been described (29,31,35,36).

Common scab is caused by soil-inhabiting gram-positive filamentous bacteria in the group known as streptomycetes. Of the hundreds of species of soil streptomycetes, few are known to be pathogenic on plants. At least 10 different species of *Streptomyces* and related *Kitosatospora* can produce

common scab in potato (3,11,23,31), and at least three additional *Streptomyces* spp. produce potato netted or russet scab (3,12). Streptomycetes causing potato scab are morphologically and phylogenetically diverse, differing in numerous morphological and physiological characteristics (4,5,13,23,24,28,31), 16S rDNA sequences (3,5,11,23,41), and cellular fatty acid profiles (22,28), and differing extensively in restriction fragment length polymorphism and repetitive element–polymerase chain reaction (PCR) patterns (11,38).

Scab diseases of underground tuber or root crops are the most widely recognized disease symptoms caused by streptomycetes, but fibrous root rots, root tumors, and phytotoxic effects on seedlings have been described (3,10,21,26,31,32). Published cross-species data are limited but it is thought that the same streptomycetes are pathogens on all host root and tuber crops, including radish (15), with the apparent exception of sweet potato (10,31). Streptomycetes isolated from potato scab lesions previously have been shown to infect radish (14,15,27), though one isolate from radish was not pathogenic on potato (27). Differences in the aggressiveness of individual *Streptomyces* isolates have been described in both potato and in radish (*Raphanus sativus*) (2,14,19,33), although inoculation densities seldom were re-

ported. There is little available information on the mechanisms of variation in pathogen aggressiveness, or how this impacts the incidence or severity of common scab disease in the field.

Most plant-pathogenic streptomycetes make at least one of a group of phytotoxins, called thaxtomins (1,10,25,31). The cyclic dipeptide toxin thaxtomin is considered a pathogenicity determinant. Genes for thaxtomin biosynthesis are located in a region of the *Streptomyces* chromosome distinguished by a different G/C composition (6–8), and hence reminiscent of a pathogenicity island (PAI). The PAI is not completely defined in *Streptomyces* spp., but is proposed to include a gene for a putative pathogenicity factor (*nec1*) at one end, and genes encoding biosynthesis of thaxtomin at the other end (*txtAB* and *txtC*), several hundred kilobase pairs distant (9). In *S. turgidiscabies*, this region appears to extend more than 400 kb. A mechanism for spread of plant pathogenicity via the horizontal transfer of the PAI into distantly related streptomycetes to produce new plant-pathogenic strains recently has been proposed (6,8).

The main objectives of this study were to (i) compare the pathogenicity (disease-causing potential) and virulence or aggressiveness (severity of disease caused) of individual *Streptomyces* isolates collected from scabby potato plants and (ii) associate aggressiveness of pathogenic isolates with known molecular disease determinants, including the presence of the *nec1* and *txtA* genes. The longer-term goals of this research are to gain insight into why the severity of disease is different from one disease-causing *Streptomyces* strain to another, and to gain information about pathogenic isolates that can be used to develop methods of predicting the suitability of a field for cultivation of scab-free root and tuber crops.

MATERIALS AND METHODS

***Streptomyces* isolates and culture conditions.** The 61 *Streptomyces* isolates used in this study are listed in Table 1. Type strains of *S. scabiei* ATCC 49173, *S. scabiei* var. *achromogenes* ATCC 33281, *S. scabiei* ATCC 33282 (neotype strain), and *S. acidiscabies* ATCC 49003, all causing common scab on potato, were obtained from the American Type Culture Collec-

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Table 1. Sources and some characteristics of isolates used in this study

Strain	Source ^a	Melanin ^b	Pathogenicity ^c	nec1 ^d	txtA ^e
ATCC 33281 <i>Streptomyces scabies</i> var. <i>achromogenes</i>	ATCC (Soil)	–	Yes	P + S + (Pst 6.5, Bam 23)	S + P +
ATCC 33282				P +	P +
<i>S. scabies</i> (neotype)	ATCC (Soil)	+	Yes	S + (Pst 6)	S +
ATCC 49003				P +	P +
<i>S. acidiscabies</i> (type)	ATCC (ME potato)	–	Yes	S + (Pst 7.5/18; Bam –)	S +
ATCC 10246 <i>S. griseus</i>	ATCC (potato)	–	No	P –	P –
ATCC 49173				P +	P +
<i>S. scabies</i> (type)	ATCC (NY potato)	+	Yes	S + (Pst 10/6.5, Bam 18)	S +
ID01-03	Not recorded, ID	–	Yes **	P + S + (Pst 6)	P + S +
ID01-6.2a	Shepody, American Falls, ID	+	D	P – S –	P + S +
ID01-8a	Russett Burbank, Burley, ID	+	Yes **	P + S + (Pst 15, Bam 18)	P + S +
ID01-9d	Shepody, Bliss, ID	–	No	P –	P –
ID01-11a	Shepody, Bliss, ID	+	No	P – S –	P – S –
ID01-11c	Shepody, Bliss, ID	–	No	P –	P –
ID01-12c	Not recorded, ID	+	Yes *	P – S –	P + S +
ID01-13c	Not recorded, ID	+	Yes	P + S + (Pst 6, Bam 21)	P + S +
ID01-14c	Not recorded, ID	–	No	P –	P –
ID01-15b	Shepody, American Falls, ID	–	No	P –	P –
ID01-15d	Shepody, American Falls, ID	–	No	P – S –	P – S –
ID01-16c	Not recorded, ID	–	Yes *	P + S + (Pst 6.5)	P + S +
ID02-6	Not recorded, Twin Falls, ID	+	Yes	P +	P +
ID02-12	Desiree; ID	+	Yes *	P – S –	P + S +
ID02-13	Yukon Gold, ID	+	No	P –	P –
ID02-14	Rose Finn Apple Fingerling, ID	+	Yes *	P +	P +
ID02-15	Red La Sota, ID	+	Yes **	P +	P +
ID02-16	Russian Banana Fingerling, ID	+	Yes *	P +	P +
MEpitB	Not recorded, ME	–	No	P –	P –
ME01-11h	B2082-1 (red), Presque Isle ME	+	Yes **	P + S + (Pst 6, Bam 23)	P + S +
ME01-12h	B2100-2 (red), Presque Isle ME	+	Yes **	P +	P +
ME01-13h	B2100-7 (red), Presque Isle ME	+	D	P + S + (Pst 15)	P + S +
ME01-14h	B2125-156 (white), Presque Isle ME	+	Yes *	P +	P +
ME01-15h	B2130-151 (russet), Presque Isle ME	+	Yes	P +	P +
ME01-16h	Ontario, Presque Isle ME	–	Yes *	P – S –	P – S –
ME01-17h	Green Mt, Presque Isle ME	+	Yes *	P +	P +
ME01-18h	B2135-170 (russet), ME	–	No	P – S –	P – S –
ME01-19h	B2133-127, Presque Isle ME	+	Yes *	P + S + (Pst 6.5)	P + S +
ME01-20h	B2133-127, Presque Isle ME	+	Yes *	P +	P +
ME01-21h	B2133-127, Presque Isle ME	–	No	P – S –	P – S –
ME01-22h	B2133-127, Presque Isle ME	+	Yes *	P +	P +
ME01-23h	B2133-127, Presque Isle ME	+	Yes *	P +	P +
ME01-24h	B2133-127, Presque Isle ME	–	No	P –	P –
MI02-2	Atlantic, E. Lansing, MI	+	Yes	P – S –	P + S +
NY02-01a	Cornell line U7S-1 Freeville, NY	+	Yes	P +	P +
NY02-01b	Cornell line U7S-1 Freeville, NY	+	Yes **	P +	P +
NY02-02a	Cornell line U7S-1 Freeville, NY	–	Yes	P +	P +
NY02-03a	Cornell line U7S-1 Freeville, NY	+	Yes **	P +	P +
NY02-7	Red La Soda, Cornell, NY	+	No	P – S –	P – S –
NY02-8	Cornell line U7S-1 Freeville, NY	+	Yes	P –	P –

(continued on next page)

^a Potato cultivar (if known), location, and state from which the scabby tuber came that was used for initial isolation of the strain.^b Production of a dark diffusible pigment, presumably melanin, on peptone-yeast extract-iron (PYI) agar.^c Measured in radish bioassay; actual severity scores in Table 2; ±, mildly pathogenic; D = whole plant died; * <50% plant survival; ** <10% plant survival.^d Presence (+) or absence (–) of *nec1* gene, determined by polymerase chain reaction (P) and confirmed by Southern blot (S); size of hybridizing band on Southern given in parentheses.^e Presence (+) or absence (–) of *txtA* gene, determined by polymerase chain reaction (P) and confirmed by Southern blot (S); hybridization was to a 6.5-kb *Pst*I fragment for all isolates positive for *txtA* on a Southern blot.

Table 1. (continued from preceding page)

Strain	Source ^a	Melanin ^b	Pathogenicity ^c	nec1 ^d	txtA ^e
NY02-18	Cornell line U7S-1 Freeville, NY	+	Yes *	P +	P +
NE01-3	Atlantic, NE	–	No	P –	P –
				S –	S –
NE02-17	Not recorded, Scottsbluff, NE	+	Yes *	P +	P +
OH01-5a	Yukon Gold, Urbana, OH	+	Yes *	P +	P +
				S + (Pst 6, Bam 23)	S +
OH01-5.2	Yukon Gold, Urbana, OH	+	Yes *	P +	P +
				S + (Pst 6)	S +
OH01-5d	Yukon Gold, Urbana, OH	+	Yes	P +	P +
				S + (Pst 6)	S +
OH01-6a	Reba, Urbana, OH	+	Yes **	P +	P +
				S + (Pst 6, Bam 23)	S +
OH01-6.2a	Reba, Urbana, OH	+	Yes	P +	P +
				S + (Pst 6)	S +
OH01-11c	B1752-5, Urbana, OH	–	No	P –	P –
OH01-12c	Reba, Urbana, OH	+	Yes **	P +	P +
				S + (Pst 6, Bam 23)	S +
WI02-1	Frito Lay numbered variety, Rhinelander, WI	+	±	P –	P –
				S –	S –
WI02-4	Russett, Plover, WI	+	Yes	P +	P +
WI02-5	Yukon Gold, Sturgeon Bay, WI	+	Yes	P +	P +
WI02-9	Not recorded, Hancock, WI	+	No	P –	P –
WI02-11	Not recorded, Rhinelander, WI	+	Yes *	P +	P +

tion (ATCC; Manassas, VA). *S. griseus* ATCC 10246 is not pathogenic on potato. Field isolates were obtained from scab lesions on potato tubers. Scabby potato plants were collected from seven states in the United States and 20 different potato cultivars. A small tuber piece containing a scab lesion was cut out and surface disinfected in 1.5% Clorox bleach for 1 to 2 min. After several rinses in sterile distilled water, the tissue was ground in a ground-glass homogenizer in 5 ml of sterile water. Serial dilutions were plated onto water agar plates (2% agar). Plates were incubated at 28°C, and individual characteristic powdery (mycelial) bacterial colonies were picked and serially transferred until a pure culture was obtained. In some cases, the original homogenate was heated for 30 min at 55°C before serial dilution and plating; this reduced the background of rapidly growing rhizobacteria. Yeast-malt extract (YME) or YME agar (39) was used for routine culture of *Streptomyces* strains. Peptone-yeast extract-iron (PYI) agar (39) was used for determination of production of dark, diffusible melanoid pigments. Colony and spore color was assessed after 2 weeks on YME plates. Strains were maintained as spore suspensions in 20% glycerol at –20 or –80°C, or as agar plugs cut from actively growing plates, and stored at –80°C.

Inoculum. A procedure modified from Goyer and Beaulieu (15) was used to produce inoculum for plant inoculation. YME (50 ml) in a 250-ml Erlenmeyer flask was inoculated with 1,000 µl of a suspension of spores harvested in water from a 2- to 3-week-old plate of *Streptomyces* spp. grown on YME agar. Spores were counted on a hemocytometer slide, and adjusted to 10⁶ CFU/ml to inoculate flasks. Cultures were grown for 3 days, with shaking, at 28°C. Cells were pelleted by centrifugation and

resuspended in dH₂O. Resuspended 3-day-old culture (8 ml) and 100 ml of sterile Say's solution (20 g of sucrose, 1.2 g of asparagine, 0.6 g of K₂HPO₄, and 10 g of yeast extract in 1 liter of dH₂O) was added to 300 ml of sterile vermiculite. Vermiculite for culture of soil inoculum was wetted with 50 ml of water per 300 ml and sterilized by autoclaving in 300-ml portions, three times for 90 min each, at 2-day intervals before inoculation with *Streptomyces* spore suspensions. Inoculated vermiculite was incubated for 14 days at 28°C and was mixed in the bags every other day during incubation. After 14 days, a 1-ml sample of vermiculite was removed, suspended in 9 ml of sterile dH₂O, incubated with shaking for 30 min, and four replicate 10-µl samples of dilutions (10^{–2}, 10^{–3}, 10^{–4}, 10^{–5}) were plated onto YME medium. Plates were incubated at 28°C for 2 days, and colonies were counted to ensure that only *Streptomyces* spp. were present in the inoculum, and to quantify and compare inoculum concentrations used in different experiments. Typically, 2.5 × 10⁸ CFU/g of vermiculite was obtained, which was diluted 1:10 (vol/vol) with sterile sand:soil for plant growth. This translates into 1.8 × 10¹⁰ CFU of *Streptomyces* spp. per pot. For the inoculum density dilution series, 14-day-old vermiculite cultures were prepared and quantified as above, and the concentration of streptomycetes was adjusted to 10⁷ CFU/g with additional sterile vermiculite. Adjusted inoculum was diluted 1:9 with sterile sand:soil for the highest inoculum concentration. One volume of this mixture then was diluted with nine volumes of sand:soil to prepare the 10⁶ CFU/g inoculum. This procedure was repeated until five 10-fold dilutions of inoculum were obtained.

Plant growth and infection. Pots (15 cm) were partly filled with 600 ml of a

50:50 mixture of sterile sand:potting soil (Jiffy Mix Plus; Jiffy Products of America, Batavia, IL). Vermiculite inoculum (300 ml) was added to 3,000 ml of 50:50 sterile sand:Jiffy Mix Plus and mixed thoroughly. Inoculum-containing mixture (1,200 ml) then was overlaid in each pot. Radish seed (*Raphanus sativus* cv. Cherriette F1; Johnny's Selected Seeds, Albion, ME) were surface sterilized (0.5% bleach for 3 min), rinsed several times in sterile distilled water, and planted directly into the inoculum-containing layer, 9 to 10 seeds per pot. Radish plants were grown in a Conviron growth chamber at 24°C, with a 14-h photoperiod, 200 µmoles m^{–2} under Philips natural daylight fluorescent lights. Plants were thinned to nine plants per pot as evenly spaced as possible, and watered when soil moisture declined to 1%, as judged by weighing the pots to determine water loss. This was approximately every 4 to 5 days, starting 1 week after germination, and becoming more frequent as plants grew. Soil started out at 50% of field capacity (about 0.4 MPa) and dried to near the wilting point (1.5 MPa), then was watered to 50% of field capacity. Radish hypocotyls were harvested and scored for scab after 5 weeks (35 days).

Disease scoring. Scab lesions were rated on all scorable radish plants after 5 weeks of growth in inoculated soil. The average number of scorable radish hypocotyls in uninoculated control plants was 15.3 (out of a possible total of 27); the corresponding average number of radish hypocotyls from soil inoculated with ATCC 49173 that could be rated in 14 experiments was 14.5. Radish hypocotyls were scored on a scale of 0 to 5, where 0 = no lesion, 1 = discrete superficial lesions less than 10 mm in diameter, 2 = coalescing superficial lesions more than 10 mm in diameter, 3 = raised lesions less than 10

mm in diameter, 4 = coalescing raised lesions more than 10 mm in diameter, and 5 = pitted or sunken lesions. All radish hypocotyls with a diameter at the widest point larger than 0.5 cm were rated, and an average calculated; each pot was considered an experimental unit. Data presented are averages of all replicate pots in an experiment, or the average of averages of all experiments done with a single isolate. The y-error bars are the average standard deviation of all experiments with an isolate, and

show the experimental variability. There are y-error bars only for isolates tested in more than one independent experiment; therefore, there is no error bar for some isolates that were tested in separate experiments if scorable radish hypocotyls were not obtained in more than one experiment. Isolates producing an average lesion severity score of less than 1.0 were considered nonpathogenic on radish. A score of 6 was assigned to isolates that killed all radish plants in an experiment for

graphic display, but this score was not used in calculating standard errors. In most cases, data are averaged from two experiments, each containing three replicate pots of nine plants. Data for 21 of the 61 isolates evaluated came from single experiments (three replicate pots, nine plants each). Data for the inoculum dilution series came from two experiments, each experiment with three isolates inoculated onto four replicate pots of nine plants each; strain ATCC 49173 was used in both

Fig. 1. Individual *Streptomyces* isolates differentially affect radish emergence, survival, and scab lesion severity. Disease parameters and inoculation densities used for each isolate are listed in Table 2. **A**, Variation in plant appearance and survival 5 weeks after sowing in inoculated soil. Each pot was inoculated with a different *Streptomyces* isolate, and planted with nine radish seeds. Isolates from upper left across to right (1–3), middle left to right (4–6), and bottom left to right (7–9) are 1, not inoculated; 2, MI02-1; 3, ATCC 49173; 4, ID02-6; 5, WI02-11; 6, ID02-12; 7, ID02-13; 8, ID02-14; and 9, ID02-15. **B**, Close up of healthy noninoculated plants. **C**, Close up of radish planted in soil inoculated with isolate ID01-8a. Plants germinated and grew for 2 to 3 weeks, then died. **D–K**, Appearance of radish plants inoculated with different *Streptomyces* isolates. Plant leaves appeared healthy in D through I. Plant leaves were necrotic, stunted, dying, or dead in J and K. D and E show radish with no scab disease. D, uninoculated; E, ID01-11a. F and G show radish plants that developed normally but had scab lesions; F, *S. scabiei* ATCC 49173; G, *S. acidiscabies* ATCC 49003. H and I show radish plants that were inhibited in their development and had scab lesions; H, ID01-13c; I, ME01-17h. J and K show severely affected radish hypocotyls from plants that were stunted, necrotic, and dying or dead. J, OH01-12c; K, OH01-5a.

experiments to verify consistency between experiments. Data from the water-treated plants (uninoculated control) are averages of 15 experiments, and data from strain ATCC 49173 are averages of 14 experiments.

Determination of the presence of the *necl* and *txtA* genes and their genomic context. For DNA isolation, *Streptomyces* cultures were grown at 28°C for 3 days in YME, and cells were pelleted and stored frozen until use. DNA was extracted from approximately 100 mg of frozen cells using a Bio101 FAST DNA kit (sphere plus garnet beads and CLS-Y lysing solution) and a Qbiogene Fast Prep machine (settings: speed 5.0, time 25 s). DNA was eluted from columns in 2× 50 µl of 10 mM Tris-HCl (pH 9.0), and 0.2 mM EDTA. Quantity and integrity of DNA preparations were checked on agarose gels, and suitability of DNA preparations for PCR amplification was verified by amplifying the 16S rRNA genes.

PCR primers were purchased from Integrated DNA Technologies (Coralville, IA). Sequences of primers used to amplify bacterial 16S rRNA were 16S-1F = 5'-CATTCACGGAGAGTTTGATCC-3' and 16S-1R = 5'-AGAAAGGAGGTGATCCAGCC-3' (8); to amplify *necl*, Nec1F = 5'-ATGAGCGGAACGGAAGCCCCGGA-3' and Nec1R = 5'-GCAGGTCGTCACGAAGGATCG-3' (6); and to amplify *txtA*, TxtA1 = 5'-CACGTACGCGCAGTTCAATG-3' and TxtA2 = 5'-AGATGATGTAGGCGGGACTC-3'. The *txtA* primers were selected with the help of the program Primer 3 (37) from sequence data for *S. acidiscabies* thaxtomin synthetase AB (GenBank accession number AF255732) (18). PCR reactions contained 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 0.1% Triton X-100, 1.5 mM MgCl₂ (2 mM MgCl₂ for *necl*; 1 mM MgCl₂ for *txtA*), 200 µM each dNTP, 25 pmol each primer, 2.5 U Taq DNA polymerase (Promega Corp., Madison, WI), 2 to 25 ng of DNA template (usually 1 µl of a 1:9 dilution), and MilliQ water in a final volume of 50 µl. Amplification was carried out in a Perkin-Elmer Thermal Cycler 480 or a GeneAmp PCR System 9600 programmed for one initial denaturation step at 95°C for 5 min followed by 40 or 42 cycles of denaturing at 95°C for 20 s; annealing at 55°C for 16S rRNA, 60°C for *necl*, and 48°C for *txtA* for 30 s; and extension at 72°C for 2 min, and ending with a 4°C hold.

The amplified DNA was separated on a 1.5% agarose gel to verify the size and presence of the expected 1,500-bp 16S rDNA, 700-bp *necl*, or 398-bp *txtA* fragments. PCR amplification experiments were repeated at least twice with two independently cultured and isolated DNA template preparations for each *Streptomyces* isolate.

Presence or absence of *txtA* and *necl* genes was confirmed by Southern blotting

for many isolates (Table 1). For restriction digestions, Fast Prep DNA preparations were further cleaned using a Gen-Elute PCR Clean-up Kit (Sigma-Aldrich, St. Louis) to improve restriction digestion efficiency. Eluted DNA was quantified by comparison with known quantities of λ-*Hind*III fragments on agarose gels, and equal amounts of DNA were digested with *Pst*I or *Bam*HI (Promega Corp.) according to the manufacturer's recommendations. Restriction-digested DNA preps were size separated on 0.8% agarose Tris-acetate EDTA gels, stained with ethidium bromide, and transferred by capillary action overnight to Amersham Hybond N+ filters using an alkaline transfer procedure, according to the protocol accompanying Amersham Hybond N+. Filters were baked for 2 h at 80°C and wrapped in plastic film until use. Prehybridization (2 h) and hybridization (20 to 22 h) were carried out at 65°C in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 0.5% blocking reagent (Roche Diagnostics, Indianapolis, IN), 5% dextran sulfate, 0.1% sodium dodecyl sulfate (SDS), and denatured fish sperm DNA at 50 µg/ml. After hybridization, filters were washed for 1 min in 2× SSC/1% SDS, followed by 15 min of 65°C in 2× SSC/1% SDS and 30 min of 65°C in 0.2× SSC/1% SDS.

DNA probes used were the 400-bp *txtA* and the 700-bp *necl* PCR fragments. For labeling, PCR fragments were excised and eluted from agarose gels using a Zymo-clean Gel DNA Recovery Kit (Zymo Research, Orange, CA). Isolated DNA fragments were labeled using a NEN Renaissance random primer fluorescein labeling kit. Unincorporated nucleotides were removed using Amersham Probe-Quant G50 spin columns, and chemiluminescent detection of hybridized products was carried out using antiluorescein-AP conjugate and the NEN CDP-Star Nucleic Acid Chemiluminescence Reagent, all according to manufacturer's directions. Kodak X-OMAT Blue film was exposed for 5 or 10 min to detect hybridization.

RESULTS

Description of streptomycetes isolated from scabby potato plants. Streptomycetes were isolated from scabby potato plants collected from at least 20 different potato cultivars from three locations in Idaho, five in Wisconsin, three in New York, and one location each in Ohio, Maine, Michigan, and Nebraska (Table 1). The original scabby tubers showed a range of symptoms from superficial small discrete lesions covering parts of a tuber to large, deep, coalescing raised or pitted lesions covering most or all of a tuber. The isolates obtained varied in their growth rate and habit in solid and liquid culture, colony color and morphology, color of spores, and production of diffusible pigments. They also varied in their pathogenicity on

radish (Figs. 1 and 2) and on two potato cultivars (*unpublished data*), and in the molecular features of the putative PAI.

Variation in pathogenicity and virulence on radish. Sixty-one isolates were assayed for pathogenicity by planting radish (cv. Cherriette F1) in soil inoculated with cultures of individual streptomycetes, and scoring for the type and extent of lesions on radish hypocotyls after 5 weeks of growth. Scab symptoms produced by different isolates varied from few or no lesions to brown, pitted, necrotic areas covering much or all of the surface of the radish (Fig. 1D to 1K). Lesion severity was scored from 0 to 5. There was some damage or cracking of uninfected radish hypocotyls so that radish plants from non-inoculated control pots had an average lesion severity score of 0.57 ± 0.35 ; therefore, isolates producing average lesion severity scores of less than 1.0 were considered nonpathogenic. Lesion severity was reproducible when the same isolate was tested in independent experiments, and seems to be characteristic of an isolate. Four type or neotype strains of *S. scabies*, *S. scabiei*, and *S. acidiscabies* obtained from the ATCC were included as reference strains (Table 1). These strains are known to cause common scab on potato, and all four of these strains caused scab on radish, with lesion severity scores of 3.0 to 3.4. The average lesion severity for *S. scabiei* ATCC 49173 in 14 independent experiments was 3.18.

Of the isolates tested, 43 had lesion severity scores greater than 1.0 and were classified as pathogenic, and 16 caused no discernable difference in symptoms compared with no inoculum or with ATCC 10246, a nonpathogenic *S. griseus* strain (Fig. 2A). Two isolates had average lesion scores close to 1.0, making their classification as pathogens ambiguous. Two pathogenic strains were mildly virulent, with lesion severity scores between 1.0 and 2.0. Most isolates, like the *S. scabiei* and *S. acidiscabies* strains, had lesion severity scores between 3.0 and 4.0. Scores for five isolates were between 2.0 and 3.0, and scores were greater than 4.0 for 10 isolates. All plants were killed by two of the isolates.

The isolates are arranged according to the average severity of scab lesions they cause in Figure 2A. However, the lesion severity scores shown actually are based on widely differing numbers of scorable radish hypocotyls obtained after inoculating pots with different isolates (Table 2). The reason for this is that, in addition to causing scab lesions on radish hypocotyls, *Streptomyces* isolates affected plant emergence, survival, and hypocotyl development, resulting in widely varying numbers of radish plants with hypocotyls larger than 0.5 cm in diameter that could be scored for scab.

Additional symptoms on plants. In addition to superficial, raised, or pitted scab

Fig. 2. Comparison of scab lesion severity, seedling emergence, and plant survival of radish plants grown in soil inoculated with different pathogenic *Streptomyces* isolates. **A**, Scab lesion severity scores of *Streptomyces* isolates. Lesions were rated after 5 weeks of growth; bars represent average scores of all scorable radishes. In most cases, data are averages of two or more independent experiments each containing three replicate pots of nine plants, and average standard deviation for multiple independent experiments is shown by the y-error bars. Data from the water-treated plants (uninoculated control) are averages of 15 experiments (each with three pots of nine plants), and data from strain ATCC 49173 are averages of 14 experiments. Plants were scored on a scale of 0 to 5 (see Materials and Methods). A score of 6 was assigned to the isolates that killed all radish plants in an experiment (far right two bars), but this score was not used in calculating standard errors. A representative nonpathogenic strain (ATCC 10246) and the uninoculated water control (open bar) are displayed on the left. Most pathogenic isolates contained both the *necI* and *txtA* genes (solid bars). Some pathogenic strains contained *txtA* but not *necI* (thick slanted stripes), and one pathogenic isolate contained neither the *txtA* nor the *necI* gene (horizontal stripes). **B**, Some *Streptomyces* isolates reduce radish seedling emergence. Bars represent the average percent emergence of seedlings. In most cases, data are averages from two independent experiments; the average standard deviation of multiple experiments is shown by the y-error bars. Isolates are arranged in the same order as in A, from least to most severe scab lesions. The bars are filled as in A: horizontal stripes, absence of *txtA* and *necI*; slanted stripes, *txtA* only; solid bars, presence of both *txtA* and *necI*. The open bar is a noninoculated (water) control. **C**, Some isolates reduced radish seedling survival, either by reducing seedling emergence or by killing the plants after they had grown for 2 to 5 weeks. *Streptomyces* isolates are arranged in the same order and the bars are filled as in A. The isolate furthest to the right killed all plants in two independent experiments.

lesions covering differing proportions of the surface of radish hypocotyls, other symptoms were produced by some isolates. These included inhibition of hypocotyl (radish) expansion, distortion of the

expanding hypocotyl, dwarfing and discoloration of leaves, and necrosis and death of entire plants (Fig. 1A and C). Many *Streptomyces* isolates reduced radish plant emergence or survival (Figs. 1A and 2B

and C). Fifty-eight isolates were evaluated for their effect on plant emergence. Emergence included germination plus early growth until plants appeared above the soil. In 15 independent experiments with

Table 2. Disease parameters produced by *Streptomyces* isolates and strains on radish

Strain ^a	Scorable (exp) ^b	Average			Log inoculation density ^d	
		Scab lesion score (SD) ^c	Emergence % (SD) ^c	Survival % (SD) ^c	Exp 1 (low)	Exp 2+ (high)
WI02-9	17 (1)	0.06	100.0	100.0	6.92	...
ID01-11a	17 (1)	0.29	...	...	9.05	...
ID01-15b	16 (1)	0.31	...	...	8.95	...
10246	34 (2)	0.35 (0.21)	98.1 (1.85)	98.1 (1.85)	8.28	8.62
ID01-11c	14 (1)	0.36	100.0	100.0	9.01	...
MEpitB	16 (1)	0.37	100.0	100.0	8.01	...
ID01-14c	16 (1)	0.38	...	...	9.05	...
ME01-18h	18 (1)	0.38	100.0	100.0	9.00	...
ID01-15d	31 (2)	0.38 (0.18)	92.6	92.6	8.51	8.45
ME01-24h	17 (1)	0.47	100.0	100.0	6.86	...
NE01-3	16 (1)	0.50	96.3	96.3	7.55	...
ID02-13	30 (2)	0.54 (0.07)	100.0 (0.00)	100.0 (0.00)	7.26	7.49
water	249 (15)	0.57 (0.35)	97.2 (3.30)	97.2 (3.30)	...	...
ME01-21h	35 (2)	0.59 (0.17)	98.1 (1.85)	98.1 (1.85)	9.00	8.86
NY02-7	17 (1)	0.65	96.3	96.3	8.26	...
ID01-9d	36 (2)	0.66 (0.19)	100.0	100.0	9.06	9.15
OH01-11c	33 (2)	0.72 (0.05)	94.4 (5.56)	94.4 (5.56)	8.44	8.85
WI02-1	48 (3)	0.96 (0.74)	97.5 (1.75)	97.5 (1.75)	7.37	8.94
NY02-8	10 (1)	1.10	88.9	88.9	8.63	...
OH01-6.2a	33 (2)	1.75 (1.33)	92.6 (3.70)	83.3 (12.96)	5.10	6.04
WI02-5	12 (1)	2.08	85.2	85.2	8.54	...
ID02-18	4 (1)	2.75	29.6	18.5	8.48	...
ID01-13c	29 (2)	2.78 (0.91)	96.3	96.3	5.79	7.81
NY02-01a	24 (2)	2.92 (0.09)	79.6 (20.37)	77.8 (22.22)	7.83	8.70
33281	35 (2)	2.96 (0.30)	96.3	74.1	6.69	6.74
ME01-19h	10 (1)	3.00	88.9	37.0	7.83	...
NY02-03a	2 (1)	3.00	40.7	7.4	8.37	...
OH01-6a	1 (2)	3.00	33.3 (33.33)	1.9 (1.85)	8.16	8.29
ID02-12	13 (2)	3.05 (0.05)	77.8 (14.81)	33.3 (3.70)	7.56	8.35
ID02-14	7 (2)	3.10 (0.10)	83.3 (9.26)	18.5 (7.41)	7.27	7.82
ME01-15h	27 (2)	3.17 (1.06)	88.9 (11.11)	77.8 (22.22)	7.19	8.66
49173	217 (14)	3.18 (0.70)	87.7 (12.59)	82.1 (17.03)	7.18	8.47
OH01-5a	20 (2)	3.20 (0.20)	66.7	40.7	6.34	8.48
ME01-20h	5 (1)	3.20	40.7	18.5	8.18	...
MI02-2	16 (2)	3.22 (0.29)	83.3 (1.85)	44.4 (29.63)	8.16	8.69
49003	25 (2)	3.29 (0.15)	55.6	29.6	7.24	7.28
33282	19 (2)	3.37 (0.64)	81.5 (3.70)	46.3 (16.67)	7.06	7.22
ID02-16	10 (2)	3.38 (0.38)	79.6 (9.26)	22.2 (0.00)	6.82	7.16
OH01-5d	10 (2)	3.38 (0.38)	50.0 (31.48)	27.8 (20.37)	6.76	8.43
ID02-15	5 (2)	3.40	79.6 (1.85)	9.3 (9.26)	7.52	7.79
ME01-17h	16 (2)	3.47 (0.47)	33.0	3.7	8.62	7.71
NY02-02a	18 (1)	3.50	100.0	100.0	8.14	...
OH01-5.2	9 (2)	3.50 (0.50)	68.5 (9.26)	42.6 (20.37)	7.45	8.20
ME01-22h	2 (1)	3.50	88.9	11.1	6.76	...
ME01-23h	7 (1)	3.57	92.6	37.0	6.41	...
WI02-11	12 (2)	3.62 (0.05)	66.7 (7.41)	27.8 (16.67)	8.40	8.70
ME01-12h	4 (2)	3.67 (0.67)	40.7 (29.63)	7.4 (3.70)	8.82	8.84
ID01-8a	3 (2)	3.67	51.9 (29.63)	11.1 (7.41)	8.33	8.81
OH01-12c	4 (2)	4.00	37.0	3.7	7.64	8.85
ME01-11h	1 (2)	4.00	59.3 (22.22)	1.9 (1.85)	8.04	8.05
ME01-14h	15 (2)	4.05 (0.55)	61.1 (9.26)	42.6 (24.07)	8.01	8.41
ME01-16h	16 (2)	4.08 (0.28)	70.4 (7.41)	44.4 (25.93)	4.37	7.31
ID01-16c	11 (2)	4.09	61.1 (27.78)	24.1 (16.67)	7.62	7.96
NY02-01b	3 (1)	4.14	66.7	11.1	7.76	...
WI02-4	10 (1)	4.20	77.8	66.7	7.43	...
ID01-12c	4 (2)	4.25	14.8	0.0	7.97	8.25
NE02-17	9 (2)	4.34 (1.14)	70.4 (0.00)	18.5 (3.70)	6.44	7.74
ID02-6	9 (1)	4.44	66.7	55.6	8.10	...
NY02-18	8 (1)	4.44	37.0	33.3	8.07	...
ID01-03	1 (1)	5.00	11.1	7.4	8.50	...
ID01-6.2a	0 (2)	D	9.3 (5.56)	1.9 (1.85)	8.36	8.81
ME01-13h	0 (2)	D	55.6 (18.52)	0.0 (0.00)	8.12	8.25

^a Arranged according to increasing scab lesion severity scores; first 18 isolates classified as nonpathogenic (lesion severity score <1.0).

^b Total scorable radish plants (number of independent experiments).

^c SD = standard deviation of the population in parentheses.

^d Log of the lowest and highest initial inoculum concentrations (in CFU/g of vermiculite) used in experiments.

noninoculated control plants, seedling emergence averaged 97% (Fig. 2B). Seedling emergence of radish plants exposed to isolates classified as nonpathogens based on scab lesion severity was greater than 90%, and the average was 98%. In contrast, the average emergence percentage of radish plants exposed to isolates classified as pathogens was 64%, but the range was from 9% to 100%. The average seedling emergence for *S. scabiei* ATCC 49173 in 14 independent experiments was 88%. Seedling emergence was greater than 80% in the presence of the other *S. scabiei* or *S. acidiscabies* type strains and for 14 of the pathogenic field isolates. The *S. acidiscabies* type strain (ATCC 49003) reduced seedling emergence to 55%. In general, isolates causing more severe scab lesions also reduced seedling emergence to a greater, but variable, extent, but there were a few isolates producing severe scab lesions that had no effect on seedling emergence or survival (e.g., NY02-02a and ME01-23h; Fig. 2B and C, Table 2). Two isolates severely reduced seedling emergence to 11% or less (ID01-03 and ID01-6.2a).

Some *Streptomyces* isolates caused chlorosis, necrosis, and, ultimately, death of plants that emerged and grew normally for 2 to 4 weeks (Figs. 1C and 2C). For noninoculated plants and for all nonpathogenic isolates, the average 5-week plant survival was the same as seedling emergence, 98%. For pathogenic isolates, the average 5-week survival was 35%, with a range of 0 to 100%. In some cases, there was no difference between seedling emergence and 5-week survival but, for most isolates, there was a further reduction in plant survival during 5 weeks. Survival of radish plants inoculated with *S. scabiei* ATCC 49173 was 82%, and with *S. acidiscabies* ATCC 49003, 30%. For several isolates, few or no plants survived after 5 weeks (e.g., OH01-6a, ME01-17h, OH01-12c, ID01-12c, ID01-6.2a, ME01-13h, and ME01-11h). Estimations of scab lesion severity were necessarily limited by lack of scorable radish plants for these isolates. Plant survival at 5 weeks was relatively reproducible, and seems to be characteristic of the isolate.

Hypocotyl development was sometimes impaired in inoculated radish plants with foliage that was healthy and indistinguishable from noninoculated plants (Fig. 1H and I). Other isolates produced stunted plants with chlorotic or necrotic foliage and severely underdeveloped radish hypocotyls (Fig. 1J and K).

Inoculum concentrations. An obvious question is whether the differences in severity of scab lesions, inhibition of seedling emergence, or chlorosis, necrosis, and plant death at later stages are primarily a function of the concentration of *Streptomyces* present in the soil. To examine the role of inoculum density in severity of scab

lesions, seedling emergence, and plant survival, radish plants were grown in a series of initial inoculum densities covering five logs. Five different *Streptomyces* isolates were used. These were selected based on differences in disease severity in initial experiments at high inoculum density. Strain ATCC 49173 produced an average scab lesion severity score and little reduction in seedling emergence or later plant survival. Isolate ID01-13c had a lower lesion severity score and higher plant emergence and survival than ATCC 49173. Isolate OH01-5a had a lesion severity score similar to ATCC 49173, but reduced seedling emergence and survival. Isolate ME01-11h had a higher lesion severity score and lower seedling emergence and survival than ATCC 49173, and radish plants grown in soil inoculated with isolate ID01-6.2a showed severely reduced seedling emergence and plant survival and no radish hypocotyl development. There were very few or no scab lesions (lesion severity scores of less than 1.0) in radish plants grown in pots inoculated with any of the *Streptomyces* isolates at 10^3 CFU/g, the lowest inoculum density used (Fig. 3A). Seedling emergence and plant survival were not affected at this inoculum density. At a 10-fold higher inoculum density, scab lesions were produced by all five strains (Fig. 3A). Lesions were most severe for isolate ID01-6.2a and least severe with ATCC 49173 and ID01-13c. The difference in severity became more pronounced as inoculum concentration was increased over two more logs. At inoculum densities of 10^6 CFU/g of vermiculite or higher, isolate ID01-6.2a killed the plants, and OH01-5a produced a lesion severity score of 5.0 (Fig. 3A). For strain ATCC 49173, the lesion severity score averaged 3.25 for all inoculum concentrations of 10^6 CFU/g or higher, up to 3×10^8 CFU/g, the highest inoculum concentration tested in any experiment (Fig. 3A and *data not shown*). Severity scores for the other two isolates also remained at approximately 3.0 for initial inoculum concentrations of 10^5 CFU/g or higher.

Seedling emergence also was affected by initial inoculum densities (Fig. 3B). Seedling emergence was reduced in ID01-6.2a and OH01-5a at inoculum concentrations of 10^6 or greater, and in ME01-11h and ID01-13c at 10^7 CFU/g, but an inoculum density of 10^8 was required to see a reduction in seedling emergence produced by ATCC 49173 (*data not shown*). Effects on long-term plant survival were evident at lower initial inoculum densities (Fig. 3C). ID01-6.2a reduced plant survival at an initial inoculum concentration of 10^4 CFU/g of vermiculite, and OH01-5a did so at 10^5 CFU/g. In contrast, an initial inoculum concentration of at least 10^8 CFU/g was required to see a sporadic effect on plant survival with strain ATCC 49173.

To assess the pathogenicity and virulence of the field isolates, we attempted to standardize the production of inoculum so that the same initial concentrations were used in every experiment. The average inoculum concentration was 2.5×10^8 CFU/g in vermiculite cultures. In most cases, inoculum densities in independent experiments with the same isolate differed by less than 1 log. These initial inoculum densities were high compared with the threshold inoculum densities required to produce disease symptoms in the dilution series experiments. The data from the dilution series also showed that there was no further effect of a 10-fold increase in inoculum density on scab lesion severity score when the initial inoculum density was greater than 10^6 to 10^7 CFU/g, though plant emergence and 5-week survival continued to diminish as the initial inoculum density increased. Based on these results, we expect that the initial inoculum densities used in assessing pathogenicity and virulence in the field isolates were high enough to produce the maximum severity of scab symptoms characteristic of an isolate.

The actual initial inoculum densities used are shown in Table 2. It can be seen that the most pathogenic isolates were not necessarily present at the highest inoculum densities. Some isolates that produced severe scab lesions and significantly reduced plant survival were present at the lowest concentrations (e.g., ME01-22h and ME01-23h). Most of the nonpathogenic isolates were present at high initial inoculum concentrations of approximately 10^9 CFU/g of vermiculite. Although the effect of a wide range of different initial inoculum densities was not examined for most field isolates, we conclude that the variation in lesion severity, reduction in seedling emergence, or longer-term phytotoxicity produced by the pathogenic isolates represents the difference in virulence characteristic of individual isolates, and are not likely to be an effect of inoculum density alone.

Production of melanoid pigments.

Production of melanoid pigments (melanin) often has been used as a marker for pathogenicity in soil streptomycetes (19,24), although it has been widely reported that pathogenic streptomycetes may not produce melanin, and many nonpathogenic strains do (5,13,19,23,41). In the present collection of 61 *Streptomyces* isolates tested for pathogenicity on radish, 42 produced a dark, diffusing pigment, presumably a melanin, on an iron-agar medium (Table 1). Though most (70%) nonpathogenic isolates did not produce melanin, and most (86%) pathogenic isolates did produce melanin, 4 of 17 isolates clearly nonpathogenic on radish produced melanin, 2 that were very mildly or ambiguously pathogenic produced melanin, and 5 of 42 pathogenic isolates, including

S. scabiei var. *achromogenes* ATCC 33281 and *S. acidiscabiei* ATCC 49003, did not produce melanin. Isolates producing a dark diffusible pigment on iron-agar medium also produced a dark pigment under standard liquid and vermiculite culture conditions.

Presence of the *txtA* gene and conserved structure of the thaxtomin gene region. All nonpathogenic isolates were missing genes for biosynthesis of thaxtomin (Fig. 2A–C; Table 1), as tested by PCR using primers from the *txtA* gene. All except one of the pathogenic isolates (ME01-16h) had the expected 398-bp *txtA* gene fragment. Presence or absence of the *txtA* gene was confirmed by Southern blotting for 27 isolates using a 398-bp portion of the *txtA* gene as a probe; in all cases, PCR and Southern results corresponded. In all 20 *txtA*-positive isolates examined on Southern blots, hybridization was to an approximately 6.5-kb *Pst*I fragment, roughly consistent with the reported 4.9-kb *Pst*I fragment incorporating *txtA* (18).

Presence of the *nec1* gene and structure of the *nec1* gene region. Nonpathogenic isolates did not have the *nec1* gene, while most pathogenic isolates had the expected 700-bp *nec1* gene fragment, as tested by PCR using primers amplifying the *nec1* gene (8). Presence or absence of the *nec1* gene in all of the isolates is shown in Table 1 and indicated on Figure 2. Of 43 pathogenic isolates, 5 were missing the *nec1* gene fragment. Presence or absence of the *nec1* gene was confirmed by Southern blotting for 28 isolates, using a 700-bp portion of the *nec1* gene as a probe (Table 1). In all cases, PCR and Southern results corresponded. Of 17 *nec1*-positive isolates, five different patterns of bands on Southern blots were seen when DNA was digested with *Pst*I, and three different patterns were seen with *Bam*HI-digested DNA (Table 1). Further evidence for sequence diversity in this region of the *Streptomyces* genome was obtained using PCR anchored in the *nec1* gene, or in an open reading frame located immediately 5' to the *nec1* gene, *ORFtnp* (data not shown). It appears that this region of the PAI is somewhat variable, both in its presence in pathogenic isolates and in its detailed molecular structure.

DISCUSSION

In this collection of 56 new streptomycetes isolated from scabby potato plants, there is diversity in morphology, pigment production, molecular characteristics of the PAI, pathogenicity, and virulence. Isolates differed in features traditionally associated with pathogenicity, such as production of a dark melanoid pigment, and one isolate lacks a gene needed for biosynthesis of the most thoroughly studied pathogenicity determinant, the phytotoxin thaxtomin. In all, 39 isolates were pathogenic on radish and 17 were not. We expected to

Fig. 3. Effect of initial inoculum concentration on scab lesion severity, seedling emergence, and plant survival. **A**, Scab lesion severity scores plotted against the log of the inoculum density for five *Streptomyces* strains. Plants were killed at an inoculum density of 10^6 or higher with isolate ID01-6.2a. The highest inoculum density attained with OH01-5a was 3×10^6 CFU/g of vermiculite. Strain ATCC 49173 was included in two experiments (● and ○). **B**, Seedling emergence plotted against the log of the inoculum density for the five isolates shown in A. **C**, Plant survival after 5 weeks plotted against the log of the inoculum density; isolates as in A.

recover nonpathogens as well as pathogens, because streptomycetes, mostly not plant pathogenic, are extremely abundant in soils, and some of these soil saprophytes are likely to co-inhabit scab lesions. Others have reported that 55% of streptomycetes isolated from scabby potato tuber lesions were pathogenic (27). Differences in virulence were manifested as scab lesion phenotypes on radish, including both differences in numbers and severity of lesions, and in phytotoxicity, measured as reduced emergence of seedlings or chlorosis, necrosis, and death of leaves after a few weeks of growth.

We expected that streptomycetes isolated from potato would be pathogenic on radish. Of the 34 isolates we tested for pathogenicity on both radish and potato, all were pathogenic on radish, and 33 were pathogenic on two potato cultivars (*unpublished data*). The observation that streptomycetes pathogenic on potato generally are pathogenic on radish is convenient, because it validates the use of a radish assay for rapidly assessing the pathogenicity and virulence of isolates. Radish plants grow more quickly and take less space than potato plants, and the time and effort required for the radish bioassay compares favorably with a widely used potato mini-tuber assay (1,22,25,28,30). The radish assay also provides additional data on seedling and plant toxicity.

One potential source of variability in disease incidence and severity was the initial pathogen load. An inoculum concentration series spanning 5 logs was set up to examine the importance of this. Scab lesion severity and phytotoxicity to seedlings and plants were evaluated after exposure to five *Streptomyces* isolates. At the lowest inoculum concentration, there was no disease produced by any of these isolates. Scab lesions were mild at a 10-fold higher inoculum density, became more severe as the initial inoculum density was increased over 3 logs, and then reached a plateau; further increases in inoculum density produced no further increase in lesion severity. The maximum lesion severity score was higher in one isolate (OH01-5a) that was more virulent than the *S. scabiei* type strain (ATCC 49173). At inoculum concentrations higher than the threshold for scab lesions, seedling emergence, and survival were reduced by the most virulent isolates. The concentration at which seedling survival began to decline was more than 100-fold lower for the two most virulent field isolates than for ATCC 49173 and the other less virulent isolates. Plants were killed without production of enlarged radish hypocotyls at 10^5 CFU/g of inoculum for the most virulent isolate (ID01-6.2a). We conclude that initial inoculum density is a major determinant of disease development. Our results are in accord with those of Keinath and Loria, who reported that potato tuber scab severity, as measured by

percentage of area covered, increased with the log of the inoculum concentration over a range of initial inoculum of approximately 4 logs (20). These researchers also found that rhizosphere populations of *Streptomyces* increased 2 to 3 logs during the 4-month experiments when the initial inoculum concentration was high, although this was not true at low initial inoculum concentrations. We measured initial inoculum density only, and have no data on how the inoculum density changed over the course of our 5-week experiments.

We could discern variation in the extent and severity of scab lesions, in the reduction in seedling emergence, and in later phytotoxicity when individual isolates were inoculated at high initial inoculum concentrations in the radish bioassay. The isolates studied in a series of initial inoculum densities showed maximal symptom severity at concentrations lower than those used in the comparison of field isolates; therefore, we expect that the inoculum density used was high enough to produce maximum disease symptoms for most or all of the isolates. We conclude that the differences in symptom severity we saw at high initial inoculum density reflect differences in virulence. We do not know how the inoculum densities used in our experiments relate to the density of pathogenic streptomycetes typically found in field soil.

Interestingly, this collection of isolates showed substantial phytotoxicity in the radish bioassay. Phytotoxicity could occur either early in development, affecting seedling emergence, or later in development, affecting long-term plant survival. There was no strict relationship between scab lesion severity scores and emergence or survival of plants. Some isolates that produced mildly or moderately scabby lesions greatly reduced plant emergence (e.g., ID02-18), whereas others produced severely scabby radish hypocotyls, but did not reduce seedling emergence or survival compared with noninoculated plants (e.g., NY02-02a). Emergence and survival percentages generally were reproducible in independent experiments with an isolate. The lethality of an isolate and whether plants die early or late in development appear to be characteristic features of the isolate. More research is needed to understand whether these two forms of phytotoxicity are the result of differences in pathogenicity or virulence factors in different *Streptomyces* isolates.

S. scabiei and *S. scabiei* strains probably are the most abundant pathogenic *Streptomyces* spp. in soils; they have been most frequently characterized and described (13,23,24,31). The *S. scabiei*, *S. scabiei*, and *S. acidiscabiei* strains used as references and most of the field isolates produced similar lesion severity scores of between 3.0 and 4.0. Only three isolates produced significantly more mild scab than ATCC 49173, with lesion scores less than

2.0, and 12 produced lesion severity scores greater than 4.0. The *S. scabiei* and *S. scabiei* reference strains were less typical in the degree of phytotoxicity they caused; 28 of the 39 pathogenic isolates in this collection reduced seedling emergence and plant survival more than ATCC 49173, and 34 were more phytotoxic than ATCC 33281. Though no effect of pathogenic soil streptomycetes on radish plant stand has been reported, this appears to be a possibility. Some isolates that are phytotoxic on radish also produced chlorosis or necrosis in potato foliage, and inhibited tuber set (*unpublished data*). It appears that strains much more virulent than the type species of *S. scabiei* and *S. acidiscabiei* are to be found on scabby potato plants, and that they may be more typical. Extensive sampling in selected areas will be necessary to determine whether the more virulent strains are most abundant in particular fields.

The production of thaxtomin is a highly reliable marker for plant pathogenicity in streptomycetes (17,18), and assays of thaxtomin production *in vitro* are quantitatively correlated with virulence on potato (17,22,31). All except one of the isolates that were pathogenic on radish had the *txtA* gene, one of a cluster of genes encoding enzymes for the peptide synthetase responsible for biosynthesis of this phytotoxic pathogenicity determinant. Researchers in Korea recently have reported two streptomycetes causing potato scab that did not produce thaxtomin (34).

Purified thaxtomin can reproduce necrosis and the tissue hypertrophy symptoms of common scab on potato tubers, and it also produced necrosis at the site of application on potato, tomato, beet, and raspberry leaves (16). Lack of seedling emergence produced by thaxtomin has been noted by King, and thaxtomin inhibits radish and *Arabidopsis* sp. seedling growth and development (25,26,31,40). These effects of pure thaxtomin are similar to the symptoms produced by some of our *Streptomyces* isolates on radish. However, it also has been shown by King that thaxtomin is not transported in plants, and its toxic effects are not systemic (21). Chlorosis, necrosis, wilting, and death of aboveground parts of the plant were produced by some isolates. This could be explained by thaxtomin damage to belowground portions of the plant, or by transportable phytotoxic products of *Streptomyces* infections.

Others have suggested that pathogenicity factors or determinants in addition to thaxtomin may be involved in disease etiology caused by streptomycetes. Kinkel et al. noted that 60% of the variation in disease severity on tubers was attributable to thaxtomin levels, leaving 40% to be accounted for by other factors (22). Leiner et al. (26) showed data indicating that *Streptomyces* cultures produce more symptoms on seedlings than culture supernatants,

suggesting that additional virulence or phytotoxicity components could be present. Thaxtomin apparently acts as an inhibitor of cellulose biosynthesis (21,40), but some differences in the effects of thaxtomin and of other cellulose biosynthesis inhibitors have been noted (21), particularly that cellulose biosynthesis inhibitors do not typically produce wilting. Whether all of the necrotic and phytotoxic effects of our *Streptomyces* isolates on radish plants can be attributed to thaxtomin remains to be experimentally verified.

Other putative pathogenicity factors that have been reported in streptomycetes causing common scab include production of a dark, diffusible pigment on iron-containing media (melanin), and the presence of *nec1*, a gene that converts a nonpathogenic *S. lividans* strain to a strain that can produce necrotic lesions on potato tuber discs (6–8). In our collection, melanin production was not always correlated with pathogenicity. Though 88% of pigmented streptomycetes were pathogenic, 4 of 42 pathogenic isolates lacked melanin, and isolates that did not produce melanin covered the full spectrum of scab lesion severity, seedling emergence percentage, and seedling survival percentage. Also, several nonpathogenic isolates did produce melanin. This is consistent with the observations of others that production of melanin is not a reliable indicator of pathogenicity (5,13,23,41).

The *nec1* gene has been reported to be present within a conserved genomic region resembling a PAI in the majority of pathogenic strains of *S. scabiei*, *S. acidiscabies*, and *S. turgidiscabies* (6,8). The compound causing necrosis that is produced by *nec1*-containing strains or transformants has not been identified, but it is distinct from thaxtomin (7). *Nec1* was not found in any nonpathogenic isolate in our collection, and was present in the majority of pathogenic isolates. However, the five isolates lacking *nec1* showed a spectrum of scab lesion severity, seedling emergence percentage, and seedling survival percentage, so it is not obvious what role this pathogenicity factor might play in virulence. The absence of *nec1* previously has been noted in one of a collection of 43 pathogenic *S. scabiei* isolates from the United States, Japan, Hungary, and South Africa (6), and in three pathogenic Korean isolates (34). The absence of *nec1* in 5 of 39 pathogenic isolates examined here suggests that this may be more common in the United States than has been reported. The *nec1* gene region was missing in Finnish potato scab-causing strains classified as *S. turgidiscabies* and *S. aureofaciens* based on 16S rRNA gene sequence and physiological characteristics (23). Isolates in the present collection have not been phylogenetically classified to *Streptomyces* sp. based on 16S rRNA sequences.

We have collected streptomycetes from potato scab lesions that show a range in

virulence phenotypes on radish. We found that initial inoculum density was a primary determinant of all symptoms of radish disease caused by streptomycetes. Differences in densities of the population of scab-causing streptomycetes in the soil seem likely to contribute to differences in incidence and severity of scab seen in the field. Symptoms included lesions on radish hypocotyls and phytotoxicity, both to seedlings and in older plants. The radish assay enabled us to rapidly determine the pathogenicity and virulence of field isolates. Pathogenic isolates generally had the *txtA* gene for thaxtomin biosynthesis, but an isolate apparently missing this pathogenicity determinant suggests the possibility of additional pathogenicity determinants to be discovered. Work is continuing to identify additional molecular features of the bacteria that correlate with their disease-causing potential and the severity of disease they cause. Further research on changes in pathogen populations over time, the effects of pathogen concentrations in soils, and the presence and effect of competitors or antagonists in field situations can contribute to understanding variability in scab incidence and severity. Molecular markers are being developed to facilitate tracing the spread of individual pathogenic strains and for quantifying pathogenic streptomycetes in soils.

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