A New Strain of *Streptomyces* Causing Common Scab in Potato

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ABSTRACT

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Common scab is a serious disease of potatoes (*Solanum tuberosum*) and other root and tuber crops, affecting the quality and market value of these crops. The disease is caused by gram-positive soil bacteria in the genus *Streptomyces*. A new common scab-causing streptomycete was isolated from scabby potatoes originating in southeastern Idaho. Research has supported a model of horizontal transfer of pathogenicity determinants among streptomycetes, and the new strain has hallmarks of the recently characterized *Streptomyces* pathogenicity island (PAI); it has genes encoding the synthetase for the pathogenicity determinant thaxtomin and for a second pathogenicity factor, tomatinase, although it lacks a third gene characteristic of the *Streptomyces* PAI, the *necl* gene. The new strain has a unique 16s rDNA gene sequence closely related to those of other pathogenic *Streptomyces* species. This 16s rDNA sequence was also found in isolates lacking a PAI, suggesting that the new pathogenic strain arose by horizontal transfer of a PAI into a saprophytic streptomycete. Isolates of the new strain are pathogenic on radish and potato, and are more virulent than the *S. scabies* type strain. In addition to scab lesions on potato tubers, lesions were also seen on underground stems and stolons. This new strain represents additional complexity in the pathogenic strains causing plant disease in the United States.

Additional keywords: *Streptomyces scabiei*, *Streptomyces scabies*

Common scab is a serious disease of potatoes (*Solanum tuberosum*) and other root and tuber crops, affecting the quality and thus the market value of these crops. In a recent survey of Canadian potato growers, 82% reported their crops were affected by common scab, with loss estimates averaging about \$100.00 per hectare (14). Other host plants for common scab include radish, carrot, beet, parsnip, and turnip (10,22). Symptoms of common scab include various types of raised, pitted, or superficial lesions on tubers that are usually darker or different in color than healthy tuber skin. The disease is caused by soil-inhabiting gram-positive bacteria in the genus *Streptomyces*. Although this genus is ubiquitous in soil, most streptomycetes are not plant pathogens. It has been estimated that a few percent of soil streptomycetes are pathogenic to plants (22).

Streptomyces scabies (synonym, *S. scabiei*) is the best-described species of scab-

causing streptomycete; it has recently been divided into several morphologically similar but genetically distinct species (2). At present, streptomycetes causing common scab in potato are classified into at least 10 different species (2,17,22,24), and at least three additional species cause netted scab in Europe or russet scab in North America (2,3,8,29). Another species causes scab on sweet potatoes (7).

Common scab occurs worldwide, but the incidence varies from region to region, year to year, and field to field. Growers report that potato common scab has become more severe, and occurs on potato cultivars that have previously been considered relatively resistant. There has been little investigation of possible reasons for this. Changes in incidence from year to year could be the result of differences in pathogen load in the soil as a consequence of weather-related changes in soil ecology leading to altered populations of pathogenic streptomycetes and/or competitors. Another possibility is the appearance of new scab-causing species or strains better adapted to new conditions or potato cultivars, or that have acquired different virulence characteristics.

A mechanism for spread of plant pathogenicity determinants in soil streptomycetes by horizontal transfer of a large chromosomal region containing genes for pathogenicity has recently been proposed (6,16). Phylogenetically diverse soil streptomycetes have thus presumably acquired a set of genes for pathogenicity harbored in a pathogenicity island (PAI) to become

plant pathogens. A conserved gene region with the characteristics of a PAI has been demonstrated in *S. scabies*, *S. acidiscabies*, and *S. turgidiscabies* (5,6,13,16).

During the course of a recent survey of plant-pathogenic streptomycetes isolated from common scab lesions on potatoes from several locations in the United States (31), a new strain of streptomycete was isolated from scabby potatoes originating in southeastern Idaho. The objective of the current study was to examine morphological and molecular characteristics, pathogenicity, and the phylogenetic relationship of this new plant-pathogenic streptomycete.

MATERIALS AND METHODS

Bacterial isolates. Five *Streptomyces* isolates obtained from scabby potatoes from southeastern Idaho are described here; two of these isolates were previously partially characterized (31). The procedure used for isolating streptomycetes from scabby potato tubers was described previously (31). Isolates were compared with the *S. scabies* type strain, ATCC 49173, obtained from the American Type Culture Collection in Manassas, VA. Although variation in virulence exists among *Streptomyces* field isolates and ATCC 49173 is not the most virulent isolate described in the literature (31), it was used because it is the type strain of *S. scabies*, and as such, it is both available to all and often used as a standard of comparison. Yeast-malt extract (YME) or YME-agar (28) was used for routine culture of *Streptomyces* isolates and strains. Strains were maintained as spore suspensions in 20% glycerol at -80°C , and/or as agar plugs cut from YME plates of streptomycetes in active growth and stored at -80°C .

Pathogenicity assays. Inoculum was prepared as described (31) with minor modifications. In short, 50 ml of YME in a 250-ml Erlenmeyer flask was inoculated with 4×10^6 *Streptomyces* spores harvested in water from a 2- to 3-week-old YME plate. Cultures were grown for 3 days, with shaking, at 28°C . Cells were pelleted by centrifugation and resuspended in one-third the original volume of sterile distilled water. Five milliliters of this resuspended 3-day culture and 50 ml of sterile $2\times$ Say's solution (40 g sucrose, 2.4 g asparagine, 1.2 g K_2HPO_4 , 20 g yeast extract in 1 liter of sterile distilled water) were added to a bag containing 300 cm^3 of sterile vermiculite. Inoculated vermiculite was incubated for 14 days at 28°C , and was mixed by shaking the bags every other

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day during incubation. After 14 days, a ca. 1 cm³ sample of the vermiculite culture was removed and shaken in 10 ml of sterile distilled water. Quadruplicate 10- μ l dilutions (10⁻², 10⁻³, 10⁻⁴, 10⁻⁵) were plated on YME agar. Plates were incubated at 28°C for 2 days, and colonies were counted to ensure that only *Streptomyces* was visible in the inoculum, and to estimate inoculum concentrations.

For both potato and radish pathogenicity testing, 15-cm pots were partly filled with 600 cm³ of a 50:50 mixture of sterile sand:potting soil (Jiffy Mix Plus). Vermiculite inoculum (300 cm³) containing 1 to 10 $\times$ 10¹⁰ CFU of *Streptomyces* was added to 2,700 cm³ of 50:50 sterile sand:soil and mixed thoroughly. Inoculum-containing mixture (1,200 cm³) was overlaid in each pot. Potato tubers were surface-sterilized for 2 min in 1.5% bleach/0.5% soap to eliminate streptomycetes other than the inoculated *Streptomyces* isolate, and rinsed in sterile distilled water before seed pieces were planted directly in the inoculum-containing layer. Potato cultivars used were Superior (moderately resistant to common scab) and Green Mountain (susceptible to common scab) (9,12). Certified seed potatoes of cultivars Superior and Green Mountain were obtained from Jewel Brothers (Monticello, ME), Cleaves Farms (Sangerville, ME), and Maine Farmers Exchange (Presque Isle, ME). Sterilized radish seeds, cultivar Cherriette F1 (Johnny's Selected Seeds, Albion ME), were planted 9 to 10 per pot, as described (31).

Potatoes and radishes were grown in Conviron growth chambers at 24°C with a 14-h photoperiod, 200 μ moles m⁻² s⁻¹ light

provided by Philips natural daylight fluorescent lamps. Plants were watered with 100 to 200 ml per pot every 2 to 3 days. Potatoes were harvested and scored for scab after 16 weeks. Three replicate pots per potato cultivar per isolate were included in each experiment, and two of the isolates were tested in at least two independent experiments. Radishes were harvested after four and a half weeks. Three replicate pots were included for each isolate tested. Two isolates were tested twice in independent experiments in radish, and the other three isolates were each tested once.

Scab lesion ratings. Scab lesions were rated on all scorable potato tubers (larger than 0.5 cm in diameter) after 4 months of growth. Potatoes were scored for severity of scab lesions and percentage of area covered by lesions. The lesion severity scale used was: 0 = no lesions; 1 = superficial lesions <10 mm in diameter; 2 = superficial lesions >10 mm in diameter; 3 = raised lesions <10 mm in diameter; 4 = raised lesions >10 mm in diameter; 5 = pitted lesions. The final disease rating assigned was on a scale of 0 to 500, which was calculated by multiplying the average lesion severity of all scorable tubers in a pot by the average percent area covered by lesions. Rating of scab lesions on radishes on a 0 to 5 scale was as described (31).

Molecular genetic analysis. For DNA isolation, *Streptomyces* cultures were grown at 28°C for 3 days in YME, and cells were pelleted to obtain ca. 100-mg aliquots which were stored frozen at -20°C until use. Alternatively, plugs were taken from 2- to 3-week-old YME plates of a *Streptomyces* isolate using a sterile no. 2

cork borer. DNA was isolated from frozen cells or from two to four agar plugs 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 $\times$ 50 μ l of 10 mM Tris-HCl, pH 9.0/0.2 mM EDTA. Suitability of DNA preparations for polymerase chain reaction (PCR) amplification was verified by PCR amplification of 16S rRNA genes using the primer pair 16s-1F and 16s 455-435 (Table 1). Primers used to uniquely identify plant-pathogenic *Streptomyces* species are listed in Table 1. All species-specific primer combinations were tested with template DNA from: *Streptomyces* sp. ATCC 10246 (not plant pathogenic), *S. scabies* ATCC 49173, *S. scabiei* ATCC 33281, *S. europaeiscabiei* ATCC 33282, *S. acidiscabiei* ATCC 49003, *S. turgidiscabiei* ATCC 700248, *S. aureofaciens* ATCC 700526, a plant-pathogenic *Streptomyces* isolate with the 16s rRNA sequence of *S. stelliscabiei* (NY02-3C), and ID03-2B, a nonpathogenic isolate that has 16s rRNA sequence in the variable regions identical to *S. bottropensis*.

Additional analysis of the presence of genes characteristic of the *Streptomyces turgidiscabiei* PAI as described by Kers et al. (16) was carried out by PCR, using primers specific to the thaxtomin A-thaxtomin B operon (*txtAB*), a tomatinase-like gene (*tomA*), and the *nec1* gene (Table 1). Primers for the *nec1* gene were previously described (5). PCR primers were purchased from Integrated DNA Technologies (Coralville, IA). PCR reactions were carried out in 50 μ l containing 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 0.1%

Table 1. Polymerase chain reaction (PCR) primers and conditions used

Gene	Primers	Annealing temperature (°C)	MgCl ₂ concentration (mM)	Product size (bp)	Reference
16S rDNA	16S-1F: 5'-CATTACGGAGAGTTTGATCC-3' 16S 455-435: 5'-ACTTTCGCTTCTTCCCTGCT-3'	55	1.5	458	6, 32
<i>nec1</i>	Nf: 5'-ATGAGCGCGAACGGAAGCCCCGGA-3' Nr: 5'-GCAGGTCGTCACGAAGGATCG-3'	60	2.0	700	5
<i>TxtAB</i>	TxtAB1: 5'-CCACCAGGACCTGCTCTTC-3' TxtAB2: 5'-TCGAGTGGACCTCACAGATG-3'	48	1.0	385	32
<i>TomA</i>	Tom3: 5'-GAGGCGTTGGTGGAGTTCTA-3' Tom4: 5'-TTGGGGTTGTACTCCTCGTC-3'	55	1.5	392	32
Species-specific 16s	ASE3: 5'-AACGGCCAGAGATGGTCGC-3' Scab2m: 5'-TTCGACAGCTCCCTCCCTTAC-3'	62	1.0	474	This work
	ASE3: (above) Aci2: 5'-CGACAGCTCCCTCCCAAG-3'	62	1.0	472	This work
	Aci1: 5'-TCACTCCTGCCTGCATGGGCG-3' Aci2: (above)	60	1.5	1,278	This work
Sequencing primers	16s-1F (see above)	55			6
	16s 455-435: 5'-ACTTTCGCTTCTTCCCTGCT-3'				32
	16s-3F: 5'-CTCTGGGCGGATACTGACG-3'				6
	16s-2R: 5'-CGTTTACGGCGTGGACTACC-3'				6
	16s 873-893: 5'-CCGCAAGGCTAAACTCAAA-3'				32
	16s 1346-1366: 5'-ATTGCTGCGGTGAATACGTT-3'				32
	16s 1366-1346: 5'-AACGTATTACCGCAGCAAT-3'				32
	16s-1R: 5'-AGAAAGGAGGTGATCCAGCC-3'				6

Triton X-100, 1.0–2.0 mM MgCl₂, 200 µM each dNTP, 25 pmol each primer, 2.5 U *Taq* DNA polymerase (Promega Corp.), 2–25 ng DNA template (1 µl of a 1:9 dilution), and MilliQ water. Amplification was carried out in a Perkin-Elmer Thermal Cycler 480 programmed for one initial denaturation step at 95°C for 3 min, followed by 40 cycles of denaturing at 95°C for 20 s, annealing for 30 s, and extension at 72°C for 2 min, and ending with a 4°C hold. Concentration of MgCl₂ and annealing temperature were optimized for each primer pair. Sequences of PCR primers, annealing temperatures, and MgCl₂ concentrations used in routine amplifications, and expected product sizes are specified in Table 1. Amplified DNA fragments were separated on 1.5% agarose gels and stained with ethidium bromide. PCR amplification experiments were repeated at least twice with at least two independently cultured and isolated DNA template preparations for each *Streptomyces* isolate.

Cloning and sequencing of the 16S rRNA gene. The 16S rRNA gene was amplified from DNA of isolates ID01-12c and ID01-6.2a by PCR using primers 16s-1F and 16s-1R (6). The 1,500-bp PCR product was excised and eluted from an agarose gel using a Zymoclean Gel DNA Recovery Kit (Zymo Research). DNA was cloned into Promega pGEM-T Easy. Six white colonies were picked for each, and DNA was prepared using an alkaline lysis quick prep procedure (27). Clones verified to contain the correct-sized insert were sequenced using the dideoxy-chain termination method and dye terminator labeling on an ABI Prism 310 Gene Analyzer at the USDA-ARS Floral and Nursery Crops Laboratory, or an ABI 4300 by Macrogen, Inc. (Seoul, Korea). Primers used are listed in Table 1, and included: T7 promoter primer, SP6 promoter primer, 16s-1F, 16s 455-435, 16s-3F, 16s-2R, 16s 893-873, 16s 1366-1346, 16s 1646-1366, and 16s-1R. Sequences were assembled using the Seqman subprogram of DNA Star, version 4.05. Complete nucleotide sequences were obtained for the 16S rRNA gene for two isolates, and are available from GenBank

(accession numbers DQ341443 [ID01-12c] and DQ861639 [ID01-6.2a]).

RESULTS

During a previous study of variation in streptomycetes isolated from scabby potatoes from several locations in the United States, a unique group of isolates was identified from southeastern Idaho (31). The isolates came from different years, field locations, potato cultivars, and types of scab lesions, but shared morphological characteristics (Table 2). These isolates are morphologically distinct from the type strain of *S. scabiei*, ATCC 49173, producing a reddish-brown substrate mycelium with gray aerial spores and a red-brown diffusible pigment on YME agar. Like *S. scabiei* ATCC 49173, they make an inky black diffusible melanoid pigment on PYI agar.

Pathogenicity on potato and on radish. Isolates from this group were pathogenic on radish and potato. At high soil inoculum densities, all five isolates were consistently more virulent than the *S. scabiei* type strain ATCC 49173 on radish, as indicated by scab lesion scores, reduced

seedling emergence, and reduced long-term plant survival (Table 3). Radish plants were severely stunted or died at high initial inoculum densities, which precluded development of normal radishes and thus common scab.

Pathogenicity and virulence of two of the isolates was examined and compared with that of *S. scabiei* ATCC 49173 on two potato cultivars. Cultivar Superior was more resistant to common scab than Green Mountain; this was evident in both scab lesion scores and in the appearance of tubers (Table 4; Fig. 1). At low initial soil inoculum densities below 4×10^3 CFU/cm³, few or no symptoms were seen on tubers of either potato cultivar with any of the three *Streptomyces* strains tested (Fig. 1, top row; Table 4). Coalescing red-brown scab lesions several millimeters deep were seen on Green Mountain tubers formed in soil infested with ATCC 49173 or with two Idaho isolates at 1,000× higher initial inoculum density of 1 to 5×10^6 CFU/cm³ soil (Fig. 1, second row left; Table 4). Scab lesions were fewer, isolated, and mostly superficial on less susceptible Superior tubers (Fig. 1, second row right; Table 4).

Table 3. Virulence characteristics of newly described Idaho *Streptomyces* isolates on radish compared with *S. scabiei*^T (ATCC 49173)

Isolate	Initial inoculum density ^a	Scab lesion score ^b	Seedling emergence (%) ^c	Plant survival after 5 weeks (%) ^c
ID01-12c	7.03	DD ^d	15	0
	6.65	4.33 (D ^e)	51.8	0
ID01-6.2a	7.47	DD	14	4
	7.06	DD	4	0
ID03-1A	7.49	4.0 (D)	95	14
ID03-2A	7.06	4.1 (D)	95	36
ID03-3A	7.0	3.5 (DD)	90	7
<i>S. scabiei</i> type strain ATCC 49173	6.75 ^f	3.48 ^f	88 ^f	82 ^f
[range]	[6.18-7.27]	[1.78-4.43]	[63-100]	[52-100]
(independent experiments)	(14)	(14)	(12)	(12)

^a Log CFU of *Streptomyces* isolate or strain per cubic centimeter of soil.

^b Severity × area, 0–5 scale; scores are averages for 3 pots in an independent experiment (9 plants per pot), or for the number of experiments given in parentheses for ATCC 49173.

^c Percentages are averages for 3 pots in an independent experiment (9 plants per pot), or for the number of experiments given in parentheses for ATCC 49173.

^d All plants dead (no radishes to score).

^e Many plants dead (few radishes to score).

^f Average in multiple independent experiments; the range among independent experiments is shown in brackets, and the number of experiments is given in parentheses.

Table 2. Features of isolates of a new *Streptomyces* strain from southeastern Idaho compared with *S. scabiei*^T ATCC 49173

Isolate	ATCC 49173 ^a	ID01-12c	ID01-6.2a	ID03-1A	ID03-2A	ID03-3A
Location and harvest year of original lesion	New York, 1984	Aberdeen, ID, 2001	American Falls, ID, 2001	Aberdeen, ID, 2003	Aberdeen, ID, 2003	Aberdeen, ID, 2003
Potato cultivar	Katahdin ^b	Not noted (white-skinned cultivar)	Shepody	Defender	Breeding line KSA 195-90	Ranger Russet
Lesion appearance	Not noted ^b	Brown slightly pitted	Deep pitted	Raised lesions	Small pitted lesions	Raised lesions
Substrate mycelium color	Light brown ^a	Reddish-brown	Reddish-brown	Reddish-brown	Reddish-brown	Reddish-brown
Spore color	Gray ^a	Gray	Gray	Gray	Gray	Gray
Color diffusible pigment on YME	Orange-brown ^a	Reddish-brown	Reddish-brown	Reddish-brown	Reddish-brown	Reddish-brown
<i>TxtAB</i> , <i>tomA</i> genes	+	+	+	+	+	+
<i>Nec1</i> gene	+	–	–	–	–	–

^a Characteristics of *S. scabiei* ATCC 49173 (=RL-34) from (18) or this work.

^b In original reference (18).

The Idaho isolates killed highly scab-susceptible Green Mountain potato plants planted in soil at this inoculum density, and killed Superior at a 10-fold higher inoculum density, similar to the effects seen on radishes (Table 4). *S. scabies* ATCC 49173 did not kill potato plants at the highest inoculum density tested (Table 4). When underground portions of wilting or dying plants were examined, the underground stems were deeply discolored, dry, and friable throughout (Fig 1, bottom row). These stems ultimately broke at ground level. Coalescing red-brown scab-like lesions were also visible on the underground stems of cultivar Superior grown in soil infested at high initial inoculum density with the Idaho strain (Fig 1, bottom row). Other *Streptomyces* field isolates tested on potatoes produced symptom severity ranging from that of ATCC 49173 to that of the Idaho strain. Symptoms included lesions or coalescing lesions on underground stems, stolons, and tubers, and were always more severe on the more susceptible cultivar Green Mountain (data not shown).

Molecular markers in the PAI. The Idaho isolates were tested for the presence of genes characteristic of the *Streptomyces turgidiscabies* PAI as described by Kers et al. (16). Using PCR primers designed to the *txtAB* operon encoding the synthetase for the toxin thaxtomin, a product of the expected size was amplified from all five isolates. Additional PCR primers were used to characterize the other "end" of the PAI. PCR analysis of the Idaho isolates revealed that the gene for a tomatinase homolog, *tomA*, located approximately 300 kb away from the *txtAB* genes in *S. tur-*

gidiscabies (6,16), was present in all five isolates. A primer pair amplifying the *nec1* gene, located in the PAI a few kilobases inside the *tomA* gene in *S. turgidiscabies* (16), did not produce the expected 700-bp PCR fragment in any of the five Idaho isolates.

Phylogenetic affiliation. There are three short regions of sequence variation in the 16s rRNA gene in plant-pathogenic *Streptomyces* species described to date (Fig. 2). These are the γ variable region between nucleotides 172 and 197, the α variable region between nucleotides 970 and 1012, and a third variable region centered on nucleotide 1435 (numbering according to the *S. scabies* type strain 16s rRNA gene sequence, GenBank accession number 971124). Based on these sequence variable regions, seven different sets of PCR primers were designed to amplify the 16s rDNA sequence characteristic of described plant-pathogenic *Streptomyces* species (Fig. 2; 32), including *S. scabies*, *S. europaeiscabiei*, *S. stelliscabiei*, *S. bottropensis*, *S. acidiscabies*, *S. turgidiscabies*, and *S. aureofaciens*. Lack of PCR amplification in the Idaho strains with *S. scabies*/*S. europaeiscabiei* and *S. acidiscabies* species-specific primers is shown in Figure 3A (lanes 5 through 9). Results with the other species-specific primer pairs are not shown, but no PCR product was obtained when any of these five Idaho isolates was used as DNA template. Therefore, the 16s rRNA gene was cloned and sequenced from two Idaho isolates. The sequence was 99% identical to the 16s rRNA sequences of *S. scabies*, *S. europaeiscabiei*, and *S. bottropensis*, differing only in one or more of three sequence

variable regions depicted in Figure 2 and at four other isolated nucleotide positions in the 16s rDNA sequence (data not shown). The Idaho isolates share the sequence of the α variable region (nucleotides 970 to 1012) with *S. scabies*, *S. europaeiscabiei*, *S. acidiscabies*, and *S. diastatochromogenes*, but differ from *S. scabies* and *S. europaeiscabiei* in the variable region centered around nucleotide 1435, and from *S. acidiscabies* and *S. diastatochromogenes* in the sequence of the γ variable region located between nucleotides 170 and 195. Use of the PCR primers ASE3 and scab2m followed by PCR primers aci1 and aci2 (Table 1; Fig. 2) separates the Idaho isolates from these four *Streptomyces* species (Fig. 3B and C). The Idaho isolates thus have a unique combination of 16s rDNA variable sequence regions, different from those previously described for pathogenic and nonpathogenic streptomycetes closely related to *S. scabies*.

Use of the Idaho + acidiscabies-specific PCR primer pair ASE3 and aci2 followed by the *S. acidiscabies*-specific primer pair aci1 and aci2 on a collection of *Streptomyces* isolates or strains revealed three non-pathogenic isolates sharing this combination of sequences in the γ , α and 1435 regions of the 16s rRNA gene (32). The three isolates came from different locations (and years) in Wisconsin, and from eastern Washington.

DISCUSSION

Earlier research has supported a model of horizontal transfer of pathogenicity determinants among closely related streptomycetes, as well as into distantly related streptomycetes (6,16,19,23), creating new

Table 4. Virulence of newly described Idaho *Streptomyces* isolates at different initial inoculum densities on two potato cultivars compared with *S. scabiei*^T (ATCC 49173)

Isolate	Initial inoculum ^a	Superior		Green Mountain	
		Scab score ^b	Experimental details ^c	Scab score ^b	Experimental details ^c
ID01-12c	7-7.5	DD ^d	2 expt (4 pots, 0 tubers)	DD ^d	2 expt (4 pots, 0 tubers)
	5.6	50 ± 30	1 expt (2 pots, 7 tubers)	462	1 expt (3 pots, 7 tubers)
	4.6	4.4 ± 3.6 ^e	1 expt (3 pots, 15 tubers)	131 ± 89	1 expt (3 pots, 7 tubers)
	3.6	13.2 ± 12.9 ^e	1 expt (3 pots, 11 tubers)	11.3 ± 11.8 ^e	1 expt (3 pots, 2 tubers)
	2.6	0.3 ± 0.3 ^e	1 expt (3 pots, 7 tubers)	5.3 ^e	1 expt (3 pots, 2 tubers)
ID01-6.2a	6.3-6.6	46 ± 37	2 expt (6 pots, 19 tubers)	DD ^d	2 expt (5 pots, 0 tubers)
	5.6	95 ± 49	1 expt (3 pots, 7 tubers)	157	1 expt (3 pots, 6 tubers)
	4.6	34 ± 3	1 expt (3 pots, 10 tubers)	271 ± 173	1 expt (3 pots, 7 tubers)
	3.6	21 ± 32	1 expt (3 pots, 10 tubers)	16 ± 17.5	1 expt (3 pots, 4 tubers)
	2.6	0.8 ± 1.3 ^e	1 expt (3 pots, 8 tubers)	8.7 ± 9.0 ^e	1 expt (3 pots, 5 tubers)
ATCC 49173	7-7.3	90.8 ± 35.5	3 expt (7 pots, 30 tubers)	292 ± 128	1 expt (3 pots, 25 tubers)
	6.7	80 ± 32	1 expt (3 pots, 11 tubers)	293 ± 15.9	1 expt (3 pots, 12 tubers)
	6.1-6.2	89.3 ± 69.2	3 expt (7 pots, 24 tubers)	202 ± 62	2 expt (5 pots, 18 tubers)
	5.9	67 ± 111	1 expt (3 pots, 8 tubers)	200	2 expt (3 pots, 3 tubers)
	5.6	22 ± 4.4	1 expt (2 pots, 5 tubers)	N ^f	1 expt (2 pots, 0 tubers)
	4.6	35 ± 45	1 expt (2 pots, 6 tubers)	90	1 expt (2 pots, 1 tuber)
	3.6	12.7 ± 17 ^e	1 expt (2 pots, 9 tubers)	24	1 expt (2 pots, 2 tubers)
	2.6	5.0 ± 6.2 ^e	1 expt (2 pots, 5 tubers)	3.1 ^e	1 expt (2 pots, 2 tubers)

^a Initial inoculum density, log CFU per cm³ of soil.

^b Score = lesion severity × area, 0-500 scale ± standard deviation of the mean.

^c Number of independent experiments (total number of pots, scorable tubers in all experiments).

^d Plants dead (no tubers to score).

^e Not different from noninoculated controls.

^f N = no tubers, plants healthy.

plant-pathogenic strains or species. Evidence is presented here of acquisition of pathogenicity in a new strain of *Streptomyces* in Idaho. The recipient strain appears to be closely related to *S. scabies*, *S. europaeiscabiei*, and other plant-pathogenic streptomycetes as defined by the sequence of the 16S rRNA gene; it is identical to one or more previously described plant-pathogenic species in each of three variable sequence regions, but has a unique combination of the three. Identification of *Streptomyces* isolates from eastern Washington and from Wisconsin with this combination of sequence variable regions in the 16S rRNA gene, but lacking genes characteristic of the PAI, suggests that the

origin of this Idaho strain could have been horizontal transfer of the PAI into a naturally occurring nonpathogenic streptomycete.

The Idaho strain shares features of the PAI with other described plant-pathogenic streptomycetes; specifically, it has the *txtAB* gene cluster and a tomatinase gene (*tomA*), which roughly delimit the “ends” of the PAI known from *S. turgidiscabiei*. However, the *necl* gene, which is located within a 425-kb ASEL restriction endonuclease fragment that also contains both the *txtAB* gene and the *tomA* gene in *S. turgidiscabiei* (16), is missing in all five isolates from Idaho. Absence of a gene homologous to *necl* was confirmed in two of

the isolates by Southern blotting using the 700-bp *necl* PCR fragment as a probe (31). Both *tomA* and *necl* are present in *S. scabies*, *S. acidiscabiei*, and *S. turgidiscabiei* (6,16). The Idaho isolates thus appear to share a version of the PAI that differs from the PAI in other plant-pathogenic *Streptomyces* species. *Streptomyces* missing the *necl* gene but retaining plant pathogenicity have previously been reported (5,17,25,31); the presence or absence of the tomatinase gene has not been reported in previous studies. Since *tomA* is located outside of *necl* in the PAI of *S. turgidiscabiei*, the presence of *tomA* in the absence of *necl* is not simply explained by incomplete conjugal transfer of the *S. tur-*

Fig. 1. Symptoms on underground parts of potato plants grown in soil infested with different isolates of plant-pathogenic *Streptomyces*. Upper left quadrant, tubers of cultivar Green Mountain, grown in soil infested with 4×10^3 (above) or approximately 10^6 (below) CFU *Streptomyces* per cm^3 soil. Left panels infested with *S. scabies* ATCC 49173, right panels with ID01-12c. Upper right quadrant, tubers of cultivar Superior, grown in soil infested with 4×10^3 (above) or 10^6 (below) CFU *Streptomyces* per cm^3 soil. Left panels infested with *S. scabies* ATCC 49173, right panels with ID01-12c. Lower left quadrant, underground parts of Green Mountain plants, grown in soil infested with 4×10^3 (above) or 10^6 (below) CFU *Streptomyces* per cm^3 soil. Left panels infested with *S. scabies* ATCC 49173, right panels with ID01-12c. Lower right quadrant, as in lower left quadrant, except the cultivar was Superior.

gidiscabies PAI. Based on morphological characteristics and the unique sequence of the 16s rRNA gene and combination of markers for the PAI, the Idaho strain appears to be a new genetic type of plant-pathogenic *Streptomyces*.

Variation in virulence of individual *Streptomyces* isolates is well-known (2,4,17,31), although comparative analyses of virulence have often failed to take into account the importance of inoculum density. At sufficient inoculum density, all five Idaho isolates were found to be pathogenic in radishes, and two isolates tested were found to be pathogenic in two potato cultivars. In radishes, the Idaho strain caused seedling lethality at high inoculum densities. Although seedling lethality was not seen at comparable inoculum densities of

the *S. scabies* type strain (ATCC 49173), other field isolates of *Streptomyces* were as aggressive as the Idaho isolates on radish, and some caused stunting of plants and seedling mortality (31). The disease caused by the Idaho isolates affected underground potato tissues in addition to tubers. Necrotic lesions were seen on stolons and the underground main stem of both Superior and Green Mountain. Lesions were superficial on stolons, stems, and tubers of the less susceptible cultivar Superior, but at high inoculum densities, necrosis of the underground stem was so extensive in the more scab-susceptible potato cultivar Green Mountain that it disrupted the vascular tissue, killing the plants. This type of plant damage has not been attributed to *Streptomyces* in field situations, but many

Streptomyces field isolates do cause symptoms on underground parts of potato plants in addition to tubers, and damage to underground portions of potato and radish plants grown in soil infested with high inoculum densities can result in above-ground symptoms (31; L. A. Wanner, unpublished data).

The Idaho strain is clearly an aggressive potato pathogen, but we do not presently know whether the presence or predominance of this strain could be responsible for differences in common scab symptoms or severity observed in regions where it occurs. Differences in common scab symptoms have often been attributed to different *Streptomyces* species (1,11,17,23), although results from subsequent work suggest there is no significant difference in the

γ variable region

	170	180	190	200
scabies (971124)	GGATACGACACTCTCGGGCATCCGATGAGTGTGGAAGCTCCGG			
europaeiscabiei (AJ007423)	A.....			
bottropensis (D63868)	A.....			
stelliscabiei (5263074)	A..CACTA.A.....T.TG.GTG.....			
diastatochromogenes (971132)	A..CACTAGG.....TCTT.GTG.....			
acidiscabies (AB026220)	TC..T.CTG.CT....GG.CG.G-.C.....			
turgidiscabies (AB026221)	T..CT..C.TC....GG.GGTG.GT.C-.....			
ID01-6.2A	A.....			
ID01-12c	A.....			
primer scab1m	CGACACTCTCGGGCATCCGA→			
primer acid1l	TCACTCCTGCCTGCATGGGCG→			

α variable region

	970	980	990	1000	1010
scabies (971124)	TACACCGGAAACGGCCAGAGATGGTCGCCCCCTTGTGGTCGGTGTACAGG				
europaeiscabiei (AJ007423)					
bottropensis (D63868)	.CGC.....GCAT.....GC.....GTG.....				
stelliscabiei (5263074)	.CGC.....GCAT.....GC.....GTG.....				
diastatochromogenes (971132)					
acidiscabies (AB026220)					
turgidiscabies (AB026221)	CATC.....GT.....				
ID01-6.2A					
ID01-12C					
primer ASE3	AACGGCCAGAGATGGTCGC→				

1435 variable region

	1430	1439
scabies (971124)	AACCCGTAA--GGGAGGGAGCTGTGCGAA	
europaeiscabiei (AJ007423)	--.....	
bottropensis (D63868)	C.TGT-.....	
stelliscabiei (5263074)	C.T.CG.....	
diastatochromogenes (971132)	C.TGT-.....	
acidiscabies (AB026220)	C.TGT-.....	
turgidiscabies (AB026221)	C.C.CG.....	
ID01-6.2a	C.TGT-.....	
ID01-12c	C.TGT-.....	
primer scab2m	←GTAA--GGGAGGGAGCTGTGCGAA	
primer acid12	←CTTGT--GGGAGGGAGCTGTGCG	

Fig. 2. Variable sequence regions in the 16s rDNA of the new Idaho strain in comparison with other pathogenic *Streptomyces* species and groups, and the nonpathogenic *S. diastatochromogenes*. Nucleotide numbering is the same as used previously (6), and is based on the *S. aureofaciens* 16s rDNA sequence. When sequence information was obtained from GenBank, accession numbers follow the *Streptomyces* species designations in parentheses. *Streptomyces* species-specific polymerase chain reaction (PCR) primers referred to in the text are indicated.

pathogenicity of *S. scabies*, *S. acidiscabies*, *S. turgidiscabies*, and *S. stelliscabiei* (3,15,20,21). Only *S. europaeiscabiei* and *S. reticuliscabiei* have been shown to differ in plant pathogenicity. *S. reticuliscabiei* causes netted scab on the surface of tubers as well as root symptoms, and both species show differences in plant host range, including differences among potato cultivars (3,26). Experiments are underway to determine whether the Idaho isolates differ in aggressiveness or host range.

Fig. 3. Unique identification of the Idaho *Streptomyces* isolates by polymerase chain reaction (PCR) with primers specific to plant-pathogenic *Streptomyces* species. **A**, DNA templates from *Streptomyces* isolates amplified with PCR primers ASE3 and Scab2m, specific for *S. scabies* and *S. europaeiscabiei*; expected PCR product size, 474 bp. **B**, The same set of DNA templates amplified with PCR primers ASE3 and Aci2, producing a 472-bp fragment from both *S. acidiscabies* and the Idaho isolates. **C**, The same set of DNA templates amplified with the *S. acidiscabies*-specific primers Aci1 and Aci2, producing a 1,278-bp DNA fragment from *S. acidiscabies*. Lane assignments: M, Promega PCR markers (sizes indicated in bp along left side of gels); 1, *S. scabies*^T ATCC 49173; 2, isolate ID01-16c (*S. europaeiscabiei*); 3, *S. acidiscabies*^T ATCC 49003; 4, isolate ME02-6987A (*S. acidiscabies*); 5, isolate ID01-6.2A; 6, isolate ID01-12c; 7, isolate ID03-1A; 8, isolate ID03-2A; 9, isolate ID03-3A.

In summary, a new plant-pathogenic *Streptomyces* strain was isolated from scabby potatoes collected in Aberdeen and American Falls, ID, in the 2001 growing season and again in Aberdeen in the 2003 growing season. Although a pathogenic version of this strain has so far been identified only from a small region of southeastern Idaho, streptomycetes having the same 16S rRNA sequence but lacking the hallmarks of a PAI, including the *txtAB*, *necl*, and *tomA* genes, have been identified from eastern Washington, and from two locations and two different years in Wisconsin. It seems likely that an endemic saprophytic *Streptomyces* species acquired a modified version of the *Streptomyces* PAI by horizontal transfer from pathogenic streptomycetes to create a new pathogenic species or strain. This new strain adds additional complexity in the pathogenic strains available to cause plant disease in the United States.

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