Characterization of Streptomyces Causing Potato Common Scab in Korea

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ABSTRACT

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Six representative Korean strains of streptomyces (S33, S27, S71, S63, S77, and S78) that were pathogenic to potato were characterized based on phenotypic properties, analysis of 16S rRNA genes, production of thaxtomin A, and presence of *necl* and ORF*tnp* gene homologs. Strains S33 and S27 had typical characteristics of *Streptomyces scabies* and *S. turgidiscabies*, respectively, producing thaxtomin A and hybridizing to genes of *necl* and ORF*tnp*. Strain S71 produced thaxtomin A and had phenotypic and phylogenetic properties similar to those of *S. acidiscabies*, except having a greater minimum growth pH (4.5), production of a melanoid pigment on tyrosine agar, and failure to hybridize with *necl* and ORF*tnp* gene probes. In contrast, strains S63, S77, and S78 were phenotypically different from described scab pathogens. Spore colors of strains S63 and S77 were yellow-white or pale orange, respectively, with rectiflexuous chains. Strain S78 had thin and compact spores unlike typical *S. acidiscabies* (ATCC 49003). Phylogenetic analysis of strains S63, S77, and S78 based on 16S rRNA gene sequences showed low homology to that of described scab pathogens (less than 97.3, 96.0, and 96.3%, respectively). Strain S78 produced thaxtomin A, but did not have homologous sequences to *necl* and ORF*tnp* genes. Production of thaxtomin A and gene homologs of *necl* and ORF*tnp* were not detected in strains S63 and S77. All three strains grow at low pH, with minimal growth at pH 3.5 (S77 and S78) or 4.5 (S63). *Streptomyces* strains S63, S77, and S78 are novel pathogenic streptomyces adapted to acidic soil conditions in Korea.

Additional keywords: horizontal transfer, phytotoxin, potato scab

Potato common scab occurs in essentially all potato growing areas of the world (5,12–16,20,22,24,32). Although scab does not affect total yields, significant economic losses result from reduced marketability because of tuber defects. Several pathogenic *Streptomyces* species have been described to date. *Streptomyces scabies* was first described by Thaxter (1892) and repropoed by Lambert and Loria (17) as the predominant species causing common scab of potatoes. A distinct acid-tolerant species, *S. acidiscabies*, induces symptoms similar to *S. scabies* (18). *S. turgidiscabies* was described from Japan (26). This species is distinct from both *S. scabies* and *S. acidiscabies* based on DNA-DNA homology, ribosomal DNA sequences, and morphological characteristics (32). *S. europaeiscabiei* and *S. stelliscabiei* were reported to cause common scab of potato, while *S.*

reticuliscabiei was identified as a cause of netted scab in France (2).

In Korea, potato has been cultivated for more than 100 years, but common scab disease was not recognized until the mid-1980s. Since this period, following an increase in cultivation, common scab has been a major limiting factor in potato production. *S. scabies*, *S. turgidiscabies*, and *S. acidiscabies* have been identified as common scab pathogens in Korea (13–15). However, *Streptomyces* spp. other than these three species also have been isolated from scab lesions on potato tubers produced in the major potato growing area in Korea. The characteristics of these organisms clearly differ from those of known scab pathogens.

The purpose of this work was to characterize major common scab pathogens in Korea based on morphological and physiological characters, analysis of 16S rRNA genes, production of thaxtomin A, and presence of *necl* and ORF*tnp* gene homologs.

MATERIALS AND METHODS

Bacterial strains and growth conditions. A total of 56 *Streptomyces* strains were isolated from potato scab lesions on field-grown potato tubers from different geographic locations using the method

previously described (8,19,27). Strains were grouped into six distinct clusters based on morphological characteristics (17,18). Six representative strains were selected for use in this study (Table 1). The type strains of three pathogenic strains (*S. scabies* ATCC 49173, *S. acidiscabies* ATCC 49003, and *S. turgidiscabies* ATCC 700248) and one nonpathogenic strain (*S. lividans* ATCC 19844) were obtained from the American Type Culture Collection (University Boulevard, Manassas, VA).

Bacteria were routinely grown at 28°C on yeast-malt extract (YME) medium (28) containing (per liter) 10 ml of the antibiotic stock solution (nystatin, 500 mg; polymyxin B sulfate, 50 mg; sodium penicillin-G, 10 mg; cycloheximide, 500 mg per 100 ml). The strains were lyophilized and stored at 4°C.

Pathogenicity tests. Strains were tested for pathogenicity based on the method of Loria and Kempter (25) with some modifications. Inoculum was prepared by adding 5 ml of sterile water to sporulating cultures growing on YME agar plates. Two milliliters of the spore suspension was added to Erlenmeyer flasks (500 ml) containing 50 ml of potato dextrose broth (PDB, Difco Laboratories, Detroit, MI); cultures were then incubated for 2 weeks at 28°C. Sterile distilled water (250 ml) was added to each flask. Potato (*Solanum tuberosum* L.) tubers of the scab-susceptible cultivar Daejima produced from greenhouse-grown plants and free of scab lesions were surface-sterilized in 0.6% NaOCl for 10 min, rinsed in distilled water, and planted in pots (19 cm diameter) containing sterilized sand. Seed pieces were thoroughly drenched with 40 ml of the inoculum. Plants were fertilized weekly with a soluble fertilizer. Greenhouse temperatures were about 24 to 29°C. Three seed pieces were placed in each pot, which was considered to be an experimental unit. Plants produced from seed pieces were harvested about 50 days after inoculation. All tubers in each pot were evaluated for symptoms of potato scab. Pathogenicity tests for all strains were repeated twice.

Pathogenicity was also tested on tuber slices (23). Cultures of test strains were grown on oatmeal agar plate (24) for 5 to 7 days at 28°C, and agar plugs from the sporulating colonies were inverted onto disks of excised tuber tissue. Disks were

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incubated in a moist chamber at 28°C in the dark and evaluated for the presence of necrosis after 5 days. All tuber slice assays were repeated three to five times.

Physiological and morphological characterization. Spore color and colony color on YME media after 14 days of incubation at 28°C were examined and compared with those described in "Methuen Handbook of Colour," 1983, reprinted by Methuen London Ltd. (17,18,29). Chain morphology was observed microscopically, and ability to produce melanin on tyrosine agar and peptone agar media was determined after 4, 8, and 12 days of incubation at 28°C (29). Sugar utilization by the strains as recommended by the International *Streptomyces* Project (ISP) was tested on basal medium (29). The lower pH limit that allowed growth was determined on YME adjusted to pH 3.0 to 7.0 at 0.5-unit intervals (17,18). The toxicities of inhibitory compounds were determined on modified Bennett agar (17).

Polymerase chain reaction (PCR) amplification. The PCR primers for 16S rRNA gene were chosen by analyzing the

previously published 16S rRNA sequences of *S. scabies*, *S. acidiscabies*, and *S. turgidiscabies* (31). Targeted DNA regions were amplified by using the following primer sets: 5'-TCACGGAGAGTTTGA-TCC-3' corresponding to positions 3 to 21 of *S. griseus* subsp. *griseus* 16S rRNA gene, and 5'-AGAAAGGAGGTGATC-CAGCC-3' corresponding to positions 1511 to 1530 of *S. griseus* subsp. *griseus* 16S rRNA gene. Amplification was carried out with 5 pmol of each primer and *Taq* polymerase (2.5 U; Promega, Madison, WI) under the following conditions: denaturing at 95°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 2 min for 30 cycles. The amplified fragments were then sequenced directly by cycling sequencing by using an ALFred autocycle sequencing kit with M13 forward and reverse primers. The sequences of the 16S rRNA genes of *Streptomyces* were compared with other *Streptomyces* from the GenBank databases.

The *nec1* and *ORFmp* genes were amplified using the previously published PCR primers Nf-Nr and Tf-Tr, respectively (3).

The reaction mix contained 50 pmol of each primer set, 200 µM dNTP, 20 mM Tris-HCl (pH 8.0), 100 mM KCl, 0.1 mM EDTA, 1 mM dithiothreitol (DTT), 50% glycerol, 0.5% Tween 20 and 0.5% Nonidet P40, 2.5 units of *Taq* DNA polymerase (Promega), and 50 ng of total DNA under the following conditions: DNA denaturing at 95°C for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 2 min for 30 cycles. The amplified DNA was then run on 1% agarose gels and repeated twice.

Phytotoxin assay. Phytotoxins were extracted from OMB inoculated with each *Streptomyces* strain and potato slices inoculated with spores as described by Babcock et al. (1) and Toth et al. (33). Briefly, oatmeal broth (100 ml) was inoculated with *Streptomyces* strains and incubated at 28°C on a rotary shaker (150 to 180 rpm) for 5 to 7 days. The cells were removed by centrifugation, and the supernatant samples were extracted three times with equal volumes of chloroform. The chloroform extracts were dried under vacuum using a rotary evaporator and then resuspended in acetone for detection of thaxtomin A by

Table 1. *Streptomyces* strains used in this study, their accession numbers of 16S rRNA genes, pathogenicity on whole plant and tuber slices, thaxtomin A production by TLC and HPLC analysis, and hybridization to *nec1* and *ORFmp* genes

Strains ^a	Accession nos. of 16S rRNA genes	Pathogenicity ^b		TLC ^c	HPLC ^d		Hybridization ^e	
		Pot	TB		OA	TB	<i>nec1</i>	<i>ORFmp</i>
<i>S. scabies</i>								
ATCC 49173 ^T	D63862	+	+	+	+	+	+	+
S33	AF361781	+	+	+	+	+	+	+
ATCC33282	D63863	NA	NA	NA	NA	NA	NA	NA
SNS-26	D63864	NA	NA	NA	NA	NA	NA	NA
<i>S. turgidiscabies</i>								
ATCC 700248 ^T	D63866	+	+	+	+	+	+	+
S27	AF361782	+	+	+	+	+	+	+
<i>S. acidiscabies</i>								
ATCC 49003 ^T	D63865	+	+	+	+	+	+	+
S71	AF361783	+	+	+	+	+	—	—
<i>Streptomyces</i> spp.								
S63	AF361784	+	+	+	—	—	—	—
S77	AF361785	+	+	+	—	—	—	—
S78	AF361786	+	+	+	+	+	—	—
<i>S. lividans</i>								
TK21	Y00484	—	—	—	—	—	—	—
<i>S. bottropensis</i>								
ATCC 25435 ^T	D63868	NA	NA	NA	NA	NA	NA	NA
<i>S. neyagawaensis</i>								
ATCC 27449 ^T	D63869	NA	NA	NA	NA	NA	NA	NA
<i>S. diastatochromo-genes</i>								
ATCC 12309 ^T	D63867	NA	NA	NA	NA	NA	NA	NA
<i>S. setonii</i>								
ATCC 25497 ^T	D63872	NA	NA	NA	NA	NA	NA	NA
<i>S. griseus</i> subsp. <i>griseus</i>								
KCTC 9080	M76388	NA	NA	NA	NA	NA	NA	NA
<i>S. sampsonii</i>								
ATCC 25495 ^T	D63871	NA	NA	NA	NA	NA	NA	NA
<i>S. eurythermus</i>								
ATCC 14975 ^T	D63870	NA	NA	NA	NA	NA	NA	NA
<i>S. tendae</i>								
ATCC 19812 ^T	D63873	NA	NA	NA	NA	NA	NA	NA
<i>S. coelicolor</i>								
A3(2)	Y00411	NA	NA	NA	NA	NA	NA	NA

^a T, type strain of the species; NA, not known or applicable.

^b Pot, pot test; TB, tuber slices; +, pathogenicity on pot test and tuber slice assays; —, nonpathogenicity on both tests.

^c TLC, thin layer chromatography; +, comigrated spot similar to authentic thaxtomin A; —, no comigrated spot similar to authentic thaxtomin A.

^d HPLC, high-pressure liquid chromatography; OA, oatmeal broth culture; TB, tuber slices; +, detection of thaxtomin A; —, no detection of thaxtomin A.

^e +, hybridization; —, no hybridization.

thin layer chromatography (TLC) and high-pressure liquid chromatography (HPLC).

Thaxtomins were also extracted from potato tuber slices inoculated with spores of each strain as described by Lawrence et al. (21). The inoculated potato tuber slices were soaked in methanol for 24 h and then homogenized for 1 h using an ultrasonic cleaner (Branson Ultrasonics Co., Danbury, CT). Cell debris and medium were removed by filtration, and the filtrate was extracted twice with equal volumes of chloroform. The chloroform extracts were dried using a rotary evaporator and dissolved in acetone for detection of thaxtomin A by HPLC.

Thaxtomins were observed on 0.25 mm Merk silica-gel 60 F₂₅₄ precoated TLC in chloroform:methanol (9:1). The bands comigrating with authentic thaxtomin A standard were recorded. Thaxtomin A was identified by HPLC using a Shimadzu 10A system with a symmetry C18 column (Waters, 3.9 × 150 mm). Thaxtomin A was eluted with 25% acetonitrile over 20 min and monitored at 380 nm.

Southern blot analysis. Total genomic DNA was digested with *Bam*HI, separated by agarose gel electrophoresis, transferred to Hybond-N⁺ nylon membranes by the manufacturer's capillary blotting method (Amersham Pharmacia Biotech, Little Chalfont Buckinghamshire, HP7 9NA,

UK), baked at 80°C for 2 h, and hybridized to random primed DNA labeling with digoxigenin-dUTP labeled DNA probes made with the DIG DNA labeling and detection kit (Boehringer Mannheim, Detroit, MI). The fragments of *nec1* (0.72 kb) and ORF*tnp* (0.55 kb) were amplified with primer sets according to Bukhalid et al. (3) and used as probes. Prehybridization and hybridization were done at 65°C for 3 h and overnight, respectively, in the following buffer: 5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 1% blocking reagent, 0.1% *N*-lauroylsarcosine, and 0.02% sodium dodecyl sulfate (SDS). The most stringent wash was 2× SSC and 0.1% SDS at room temperature for 2 × 5 min and 0.1× SSC and 0.1% SDS at 65°C for 2 × 15 min. DIG-labeled probes were then detected following the manufacturer's suggested methods (Boehringer Mannheim).

RESULTS

Pathogenicity. All six Korean strains induced scab lesions on tubers (Table 1, Fig. 1). Tuber lesions produced following inoculation with each isolate were similar and identical to those from scab lesions on field-grown tubers. In addition, pathogens reisolated from inoculated progeny tubers had spore color and morphology identical to those of the original pathogens. In tuber

slice assays, all six strains induced necrosis on tuber slices (Fig. 1).

Phenotypic characterization. Strains were compared for substrate mycelium and spore color in mass, spore chain morphology, melanin production, sugar utilization, and resistance to inhibitory compounds (Table 2). Strain S33 was identical to the type strain of *S. scabies*, except that it failed to grow in the presence of 5 to 6% NaCl and was resistant to 10 IU/ml penicillin. Strain S27 was identical to the type strain of *S. turgidiscabies* except that it failed to produce melanin on peptone agar and was resistant to 10 IU/ml penicillin and 25 µg/ml oleandomycin. Strain S71 had characteristics similar to those of *S. acidiscabies*, except that its growth was inhibited below pH 4.5 and it produced melanin on tyrosine agar and varied in its reaction to several antibiotics and other inhibitory compounds. Strain S63 produced yellow-white spores in a rectiflexuous chain, produced melanin on tyrosine or peptone agar, and utilized all the International *Streptomyces* Project (ISP) sugars. Growth of this strain was inhibited below pH 4.5 and in the presence of 5, 6, or 7% NaCl, thallium acetate (100 µg/ml), phenol (0.1% vol/vol), oleandomycin (25, 100 µg/ml), and streptomycin (20 µg/ml). Strain S77 had pale orange spores in a rectiflexuous chain, produced melanin on

Fig. 1. Necrotic halos produced around agar plugs from 5-day-old oatmeal agar cultures of *Streptomyces* species (left) and lesions produced on potato tubers (right). A, S33, B, S27, C, S71, D, S63, E, S77, F, S78, and G, *S. lividans* ATCC 19844.

tyrosine agar, and utilized all the ISP sugars. Growth was inhibited below pH 3.5 and in the presence of thallium acetate (10, 100 µg/ml) and streptomycin (20 µg/ml). Strain S78 had thin and compact spores in a rectiflexuous chain, did not produce melanin on tyrosine or peptone agar, and utilized all ISP sugars. The growth was inhibited below pH 3.5 and in the presence of 5, 6, or 7% NaCl, thallium acetate (10, 100 µg/ml), crystal violet (0.5 µg/ml), phenol (0.1%), oleandomycin (25, 100 µg/ml), and streptomycin (20 µg/ml). The morphological and physiological characteristics of the three strains (S63, S77, and S78) differ from those of described scab pathogens, such as *S. scabies*, *S. turgidiscabies*, and *S. acidiscabies* (Table 2).

Analysis of 16S rRNA genes. The complete sequences of the 16S rRNA genes of six strains causing potato scab disease were determined (Table 1, Fig. 2). Their sequences were compared with those in GenBank. Strain S33 was most similar to *S. scabies* strains (ATCC 49173, ATCC 33282, SNS-26) with sequence identities of more than 99.8%. Phylogenetic analysis confirmed clustering of S33 with *S. scabies* strains in 100% of bootstrap replicates (Fig. 2). Strain S27 was most similar to *S. turgidiscabies* ATCC 700248 with sequence identities of 99.5%, and clustering was supported by 100% of bootstrap repli-

cates (Fig. 2). Strain S71 was identified as most similar to *S. acidiscabies* ATCC 49003 with sequence identities of 99.9%, and these clusters were also supported by 100% of bootstrap replicates. In contrast, strain S63 had less than 96.0, 97.2, and 97.3%, strain S77 had less than 95.2, 95.5, and 96.0%, and strain S78 had less than 95.5, 96.1, and 96.3% similarities to three type strains of *S. scabies*, *S. turgidiscabies*, and *S. acidiscabies*, respectively.

Analysis of thaxtomin A production. Extracts of oatmeal broth culture of *S. scabies* (ATCC 49173 and S33), *S. turgidiscabies* (ATCC 700248 and S27), *S. acidiscabies* (ATCC 49003 and S71), and Korean strains (S63, S77, and S78) produced a yellow band that comigrated with authentic thaxtomin A by TLC analysis (Table 1). HPLC analysis of chloroform extracts from oatmeal broth and potato slices inoculated with spores showed a peak of thaxtomin A from all strains except S63 and S77.

PCR analysis of *necl* and *ORFmp* genes. The predicted 720- and 550-bp products for the coding sequences of *necl* and *ORFmp*, respectively, were obtained from *S. scabies* ATCC 49173 and strain S33, *S. turgidiscabies* ATCC 700248 and strain S27, and *S. acidiscabies* ATCC 49003 using the originally described primer sets (3). No precise product was

obtained with the two primers from the pathogenic strains S71, S63, S77, and S78 and a nonpathogenic strain *S. lividans* ATCC 19844 (Fig. 3).

Southern blot analysis of the *necl* and *ORFmp* genes. The *necl* probe hybridized to a single fragment (>9.4 kb) of genomic DNA from *S. scabies* (ATCC 49173 and S33) and *S. turgidiscabies* (ATCC 700248 and S27) and to a single fragment (<5.0 kb) of genomic DNA from *S. acidiscabies* (ATCC 49003), but failed to hybridize to DNA from Korean *S. acidiscabies* strain S71, strains S63, S77, and S78, and nonpathogenic *S. lividans* ATCC 19844 (Fig. 4).

Similarly, the *ORFmp* probe hybridized to one fragment of genomic DNA (>9.4 kb) from *S. scabies* (ATCC 49173 and S33), *S. turgidiscabies* (ATCC 700248 and S27), and *S. acidiscabies* (ATCC 49003), but failed to hybridize to genomic DNA from strains S71, S63, S77, and S78 and nonpathogenic *S. lividans* ATCC 19844 (Fig. 4). The additional homologous fragment (<5.0 kb in size) from *S. scabies* ATCC 49173 hybridizing to *ORFmp* previously reported by Bukhalid et al. (3) was not observed.

DISCUSSION

This paper presents the first extensive study of pathogenic bacterial strains inducing potato common scab disease in Korea,

Table 2. Phenotypic characteristics of *Streptomyces* causing potato scab in Korea

Characteristics	S33	S27	S71	S63	S77	S78	<i>S.s</i> ^a	<i>S.t</i>	<i>S.a</i>	<i>S.c</i>	<i>S.e</i>	<i>S.st</i>	<i>S.r</i>
Spore color ^b	G	G	W	YW	PO	W	G	G	W	W	G	G	LG
Chain morphology ^c	S	Rf	Rf	Rf	Rf	Rf	S	Rf	Rf	Rf	S	S	Rf
Colony color on YME ^d	TB	RY	LY	OB	GO	LY	TB	Y	Br	GB	ND	ND	ND
Melanin on tyrosine agar	+	+	+	+	+	+	+	+	+	+	+	+	+
Melanin on peptone agar	+	+	+	+	+	+	+	+	+	+	+	+	+
Utilization													
L-Arabinose	+	+	+	+	+	+	+	ND	+	+	+	+	+
D-Fructose	+	+	+	+	+	+	+	ND	+	+	+	+	+
D-Glucose	+	+	+	+	+	+	+	ND	+	+	+	+	+
D-Mannitol	+	+	+	+	+	+	+	ND	+	+	+	+	+
Raffinose	+	+	+	+	+	+	+	+	+	+	+	+	+
Rhamnose	+	+	+	+	+	+	+	ND	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	ND	+	+	+	+	+
D-Xylose	+	+	+	+	+	+	+	ND	+	+	+	+	+
i-Inositol	+	+	+	+	+	+	+	ND	+	+	+	+	+
Minimum growth pH	5.0	4.5	4.5	4.5	3.5	3.5	5.0	4.5	4.0	5.0	ND	ND	ND
Growth with ^f													
5% NaCl	+	+	+	+	+	+	+	+	+	+	+	+	+
6% NaCl	+	+	+	+	+	+	+	+	+	+	+	+	+
7% NaCl	+	+	+	+	+	+	+	+	+	+	+	+	+
Thallium acetate	+	+	+	+	+	+	+	+	+	+	+	+	+
Thallium acetate	+	+	+	+	+	+	+	+	+	+	+	+	+
Crystal violet	+	+	+	+	+	+	+	+	+	+	+	+	+
Phenol	+	+	+	+	+	+	+	+	+	+	+	+	+
Penicillin	+	+	+	+	+	+	+	+	+	+	+	+	+
Oleandomycin	+	+	+	+	+	+	+	+	+	+	+	+	+
Oleandomycin	+	+	+	+	+	+	+	+	+	+	+	+	+
Streptomycin	+	+	+	+	+	+	+	+	+	+	+	+	+

^a Type strain of *S. scabies* (*S.s*), *S. turgidiscabies* (*S.t*), *S. acidiscabies* (*S.a*), *S. caviscabies* (*S.c*), *S. europaeiscabiei* (*S.e*), *S. stelliscabiei* (*S.st*), *S. reticuliscabiei* (*S.r*) from references Bouček-Mechiche et al. (2); Goyer et al. (9); Lambert and Loria (17,18); Miyajima et al. (26).

^b G, gray; W, white; YW, yellowish white; PO, pale orange; LG, light gray.

^c S, spiral; Rf, rectiflexuous.

^d YME, yeast malt extract; TB, tan-brown; RY, red-yellow; LY, light yellow; OB, olive brown; GO, gray-orange; Y, yellow; Br, brown; GB, golden brown.

^e +, positive reaction; -, negative reaction; v, between 20 and 90% of the strains were positive; ND, not determined.

^f Thallium acetate, 10 µg/ml, 100 µg/ml; crystal violet, 0.5 µg/ml; phenol, 0.1%; penicillin, 10 IU/ml; oleandomycin, 25 µg/ml, 100 µg/ml; streptomycin, 20 µg/ml.

and we believe that the six Korean representative strains (S33, S27, S71, S63, S77, and S78) characterized in this study are the major causal organisms of potato scab in Korea.

In these six Korean representative strains, three strains were identified as *S.*

scabies (S33), *S. turgidiscabies* (S27), and *S. acidiscabies* (S71) according to their morphological and physiological properties and their 16S rRNA sequence identity (17,18,26,29). Spore color, spore chain morphology, and pigment production of the morphological and physiological properties

appeared to be the most reliable phenotypic criteria for their identification. Physiological characteristics such as minimum pH for growth, carbon utilization, and antibiotic resistance appear to be less consistent. The three remaining strains differed in phenotypic, morphological, and 16S rRNA sequence characteristics from known common scab pathogenic species (17,18,26).

Strains S63 and S77 produced yellow-white and pale orange spores, respectively. Strain S78 had white spores with rectiflexuous chain morphology, similar to *S. acidiscabies*. However, strain S78 had thin and compact colonies, distinct from that of typical *S. acidiscabies* ATCC 49003 (18). Growth of strain S63 was inhibited below pH 4.5 and of strains S77 and S78 below pH 3.5, suggesting that all three strains might have evolved to the commonly acidic soil conditions in Korea. Similar environmental adaptations were reported in several potato-growing areas in Canada, Europe, and Israel (5,6,7,9,16,22). Therefore, we consider strains S63, S77, and S78 novel pathogenic species adapted to acidic soil condition in Korea.

Identity of the six pathogenic Korean *Streptomyces* strains based on 16S rRNA gene sequences was supported by the above grouping based on phenotypic properties. Our phylogenetic analysis was consistent with the previous reports of Takeuchi et al. (31), Kreuze et al. (16), and Bouček-Mechiche et al. (2). In this study, strain S63 exhibited the highest levels of sequence similarity with *S. setonii* (99.3%) and *S. griseus* subsp. *griseus* (99.0%), which are placed in the subcluster 1B proposed by Williams et al. (34). But *S. griseus* and *S. setonii* were reported to be most closely related to the saprophytic *Streptomyces* CFBP 4543 by Bouček-Mechiche et al. (2). In DNA-DNA hybridization studies, however, strain S63 showed little homology with *S. griseus* and *S. setonii* at 10 and 7%, respectively (unpublished data). Because DNA hybridization provides a more precise measure of the

Fig. 2. Phylogenetic tree of *Streptomyces* spp. based on the 16S rRNA gene sequences obtained by the Neighbor-Joining method.

Fig. 3. Polymerase chain reaction amplification of the **A**, *necI* (720 bp); and **B**, *ORFmp* (550 bp). M: molecular marker (bacteriophage λ DNA digested with *Hind*III), Lane 1: *Streptomyces scabies* ATCC 49173^T, 2: S33, 3: *S. turgidiscabies* ATCC 700248^T, 4: S27, 5: *S. acidiscabies* ATCC 49003^T, 6: S71, 7: S63, 8: S77, 9: S78, 10: *S. lividans* ATCC 19844. Arrows are indicated for amplified *necI* (**A**, 720 bp) and *ORFmp* (**B**, 550 bp) fragments, respectively.

level of relatedness between highly related organisms (2,30), we can conclude that strain S63 is phylogenetically distinct from other *Streptomyces* spp. The acid tolerant potato scab pathogens of Korea, strains S77 and S78, were distinct from any known *Streptomyces* spp.

To date, all strains of *Streptomyces* pathogenic to potato from diverse origins tested produce thaxtomin A (1,9,21,23, 24,33). In this study, chloroform extracts of oatmeal broth from all six Korean strains produced a yellow band, which comigrated with the thaxtomin A standard. Thaxtomin A was not detected from culture media of strains S63 and S77 by HPLC analysis. Each HPLC analysis was repeated twice; therefore, we believe failure to detect thaxtomin A production by these two strains was not due to any artifact. When chloroform extracts from tuber slices were analyzed by HPLC, again, thaxtomin A was not detected from strains S63 and S77, which clearly indicated that Korean strains S63 and S77 did not produce thaxtomin A either in the media or in potato tissues. Any pathogenic compounds produced by these two strains S63 and S77 will be characterized in a future study. Few reports on pathogenicity factors other than production of thaxtomin A, such as production of hydrolytic enzymes causing deep-pitted le-

sions, have been made previously (7,9). Pathogenic strains that do not produce thaxtomin A may be highly significant if disease resistance based on detoxification or blocking thaxtomin A toxicity is employed (Calum Wilson, University of Tasmania, Australia, *personal communication*). In this study, *necl* gene and the 5' transposase pseudogene *ORFtnp* were successfully amplified from all strains studied except for *S. acidiscabies* S71 and the three novel Korean strains, S63, S77, and S78. However, successful amplification by PCR only shows the possibility of homologous DNA sequences matched to primers. Thus, Southern blot analyses were done using probes derived from *necl* and *ORFtnp* genes which also failed to hybridize to the above four Korean strains. We, therefore, conclude that strains S71, S63, S77, and S78 do not possess *necl* or *ORFtnp* gene homologs to those in *S. acidiscabies*. These genes are present within a putative pathogenicity island associated with synthesis of thaxtomin A and induction of common scab disease (4,10,11). *necl* has no known function, while *ORFtnp* is suggested to be involved in movement of pathogenicity genes between *Streptomyces* spp. Strains S63 and S77 did not appear to produce thaxtomin A in culture, which may explain the absence

of *necl* and *ORFtnp* gene homologs in these strains. However, the absence of these two genes in strains S71 and S78 is surprising. Both genes appear dispensable for pathogenicity and thaxtomin A synthesis, and their absence could reflect the loss of nonessential genes in the transfer of pathogenicity genes of Korean strains.

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Fig. 4. Southern hybridization analysis of a *Bam*HI digest of representative *Streptomyces* strains with **A**, *necl* (720 bp) and **B**, *ORFtnp* (550 bp) used as DNA probes. Lanes a and b are *S. scabies* ATCC 49173^T and S33; c and d are *S. turgidiscabies* ATCC 700248^T and S27; e and f are *S. acidiscabies* ATCC 49003^T and S71; g, S63; h, S77; i, S78; and j, *S. lividans* ATCC 19844. Sizes in base pairs are indicated for detected fragment of the *necl* and *ORFtnp* based on reference (4).

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