Phylogenetic Analysis of 16S rRNA Genes and PCR Analysis of the *nec1* Gene from *Streptomyces* spp. Causing Common Scab, Pitted Scab, and Netted Scab in Finland

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ABSTRACT

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The sequences of the 16S rRNA genes (nucleotides 29 to 1,521) from various *Streptomyces* strains pathogenic to potato were compared. These included 10 pathogenic *Streptomyces* strains isolated from potato scab lesions in Finland, the type strains of *S. aureofaciens* NRRL 2209^T and *S. lydicus* ATCC 25470^T, '*S. griseus* subsp. *scabies*' ATCC 10246, and two *S. griseus* strains that were originally deposited to the collection as pathogens. The nucleotide sequence (>94.5% sequence identity [SI]) and length (1,469 to 1,481 nucleotides) of the analyzed region varied. Phylogenetic analysis of 16S rRNA genes placed Finnish strains into three species, supported by previously characterized morphological and physiological traits. Six Finnish strains, including two strains that deviated from the others in one trait (no spiral sporophores or D-xylose utilization), had identical 16S

rRNA genes and were identified as *S. scabies* (99.9% SI to *S. scabies* ATCC 49173). Three Finnish strains were identified as *S. turgidiscabies*, a species previously described only in Japan (99.9% SI to *S. turgidiscabies* ATCC 700248). Finnish strain 317 and *S. aureofaciens* NRRL 2209 (99.8% SI) were placed in a distinct phylogenetic cluster together with *Kitosatospora* spp., which suggests that *S. aureofaciens* may belong to the recently revived genus *Kitosatospora*. In pathogenicity tests, *S. scabies* caused characteristic symptoms of common scab, *S. turgidiscabies* caused mainly pitted scab, and *S. aureofaciens* caused netted scab and necrotic lesions on stolons of potato cultivars Bintje and Matilda in the greenhouse. The *nec1* gene and the intergenic region between *nec1* and the 5' transposase pseudogene *ORFmp* were successfully amplified by polymerase chain reaction from *S. scabies* ATCC 49173 and the pathogenic Finnish strains of *S. scabies*, but not from a nonpathogenic strain of *S. scabies*, three pathogenic and two nonpathogenic strains of *S. turgidiscabies*, and *S. aureofaciens*.

Scab diseases occur on potato worldwide and cause economically significant losses in yield (23). *Streptomyces scabies* causes a disease on potato tubers that was originally described with the name 'common scab.' The symptoms can consist of erumpent, superficial, or pitted lesions, depending on pathogen strain and cultivar susceptibility (18). Also, *S. griseus* (7), *S. cinerchromogenes* (1), *S. corchorusii* (1), *S. aureofaciens* (7), the more acid-tolerant species *S. acidiscabies* (19), and *S. turgidiscabies* (25,37) have been reported to cause symptoms similar to common scab. Although highly virulent strains of *S. scabies* can cause pitted lesions in susceptible cultivars (23), such lesions are typical for another species named *S. caviscabies* (12), which occurs under high-moisture conditions in Canada. Furthermore, Archuleta and Easton (1) reported four other *Streptomyces* spp. (*S. diastatochromogenes*, *S. atroolivaceus*, *S. lydicus*, and *S. resistomycificus*) to be causal agents of deep-pitted scab in the state of Washington. The names 'russet scab' and 'netted scab' are used on the American continent and in Europe, respectively, to describe similar superficial symptoms on potato tubers. Russet scab and netted scab have, however, been shown to differ in several characteristics such as cultivar susceptibility, root attack, and optimum soil temperature and are, therefore, considered to be different diseases and can probably be caused by several *Streptomyces* spp. (31). Some of the strains causing russet scab have been placed into the *S. aureo-*

faciens cluster of Williams et al. (39) by Faucher et al. (11). *Streptomyces* strains that cause netted scab in acidic and moist soils under cool conditions in Sweden are divided into two groups, one of which tolerates lower pH than does *S. scabies* (2). Pathogenic streptomycetes isolated from potato scab lesions in Israel belong to a phenotypically diverse population of *Streptomyces* spp. assigned to different phenotypes (8).

In a recent study (21), *Streptomyces* strains isolated from scab lesions on potato tubers in Finland were characterized for colony color, sporophore morphology, pigment production, utilization of the International *Streptomyces* Project (ISP) sugars (32), growth under acidic conditions, and pathogenicity indicated by an ability to induce necrosis on potato minitubers (5,20). Based on these criteria, most pathogenic strains were identical to *S. scabies* (18). Another large group of strains (designated group 4) distinguishable from *S. scabies* had flexuous sporophores, did not produce melanin, had tan colonies, utilized all ISP sugars, and grew at pH 4.4, in contrast to *S. scabies*, which did not grow at or below pH 4.6. Most group 4 strains were nonpathogenic. Three pathogenic strains of this group (287, 298, and 300) and one Finnish strain of *S. scabies* (SSC 101) have been tested in the greenhouse and shown to kill the sprouts of presprouted tubers of potato cultivars Bintje and Matilda, which were planted in washed sand previously inoculated with these strains (J. P. T. Valkonen, unpublished data). The whole-cell fatty acid composition of these group 4 strains clearly differs from that of *S. scabies* and *S. acidiscabies* (21). Strain 317 was able to cause necrosis on potato minitubers, but differed from the group 4 strains in that it had yellow colonies on yeast malt extract (YME) medium and did not grow at pH 4.4 (21).

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16S rRNA gene sequences are an important criterion in microbial taxonomy (33), including that of the actinomycetes (34). The aim of this study was to determine 16S rRNA gene sequences in selected Finnish *Streptomyces* strains that differ in physiological and morphological characteristics but are able to induce necrosis on potato minitubers (21). Additionally, 16S rRNA genes from five type strains of three *Streptomyces* species were sequenced in order to extend the number of plant-pathogenic *Streptomyces* spp. for which the 16S rRNA sequences are known and to permit a more comprehensive comparison between the Finnish strains and previously described species. Selected Finnish strains representing different phenetic groups were also tested for the type of symptoms they produced on potatoes in the greenhouse. Primers designed to amplify the genetic region *ORFmp-nec1*, which is linked to thaxtomin production and pathogenicity in *S. scabies* (5,6), were tested as markers for pathogenicity of Finnish *Streptomyces* strains representing three different species.

MATERIALS AND METHODS

***Streptomyces* strains.** *Streptomyces* strains analyzed in this study are listed in Table 1. The strains from Finland were isolated

from scab lesions on field-grown potato tubers from different geographic locations and were previously characterized (21). Three Finnish strains of *S. scabies*, each differing from the *S. scabies* type strain (^T ATCC 49173^T (18) by only one taxonomic trait, were also included. Strains 364 and 380 are pathogenic (21). Strain 380 produces flexuous rather than spiral sporophores on YME medium of the ISP (32), and strain 364 does not utilize D-xylose. Strain 346 is nonpathogenic and does not utilize D-mannitol. The origins of the other *Streptomyces* strains included in this study are given in Table 1.

DNA isolation. *Streptomyces* strains were maintained on potato dextrose agar (PDA) (Difco Laboratories, Detroit) at 4°C. For DNA isolation, they were grown on PDA at 24°C for 2 weeks, scraped from the PDA plate, and ground in liquid nitrogen. DNA isolation was carried out using the method provided by M. Kokkola. Frozen bacteria (200 to 500 mg) was transferred to a 2-ml microcentrifuge tube containing 500 µl of TENP buffer (50 mM Tris, pH 8.0; 20 mM EDTA, pH 8.0; 100 mM NaCl; and 1% polyvinylpyrrolidone) and 150 µl of 10% sodium dodecyl sulfate. After brief vortexing, the tube was dipped into liquid nitrogen, incubated at 100°C for 5 min and at 65°C for 60 min, and then centrifuged at

TABLE 1. *Streptomyces* and *Kitosatospora* strains analyzed in this study

Strain	Origin ^a	Group assignment ^b	16S rRNA sequence		
			Reference	Length of sequence ^c	Database accession numbers ^d
Pathogenic Finnish strains					
SSC 101	Finland	1a	This study	1,477	Y15493
267	Finland	1a	This study	1,477	Y15500
289	Finland	1a	This study	1,477	Y15509
302	Finland	1a	This study	1,477	Y15508
364	Finland; DSM 41744	1b	This study	1,477	Y15497
380	Finland	3	This study	1,477	Y15506
287	Finland; DSM 41745	4	This study	1,479	Y15495
298	Finland; DSM 41746	4	This study	1,479	Y15496
300	Finland; DSM 41747	4	This study	1,479	Y15499
317	Finland; DSM 41743	5	This study	1,469 ^e	Y15505
Nonpathogenic Finnish strains					
346	Finland	1b			
268	Finland	4			
283	Finland	4			
Reference strains					
<i>S. acidiscabies</i>	ATCC 49003 ^T		37	1,478	D63865
<i>S. ambofaciens</i>	ATCC 23877 ^T		28	1,478	AF008220
<i>S. aureofaciens</i>	NRRL 2209 ^T		This study	1,469 ^e	Y15504
<i>S. bottropensis</i>	ATCC 25435 ^T		37	1,478	D63868
<i>S. coelicolor</i>	A3(2)		3	1,478	Y00411, M35377
<i>S. diastatochromogenes</i>	ATCC 12309 ^T		37	1,479	D63867
<i>S. eurythermus</i>	ATCC 14975 ^T		37	1,478	D63870
<i>S. griseus</i> (1)	H. Schrempf, Germany		This study	1,478	Y15502
<i>S. griseus</i> (2)	ATCC 10137, R. Loria, United States		This study	1,478	Y15501
<i>S. griseus</i> subsp. <i>griseus</i>	KCTC 9080		16	1,476	X55435
' <i>S. griseus</i> subsp. <i>scabies</i> '	ATCC 10246, R. Loria, United States		This study	1,479	Y15503
<i>S. lividans</i>	TK 21		36	1,474	Y00484
<i>S. lydicus</i>	ATCC 25470 ^T		This study	1,481	Y15507
<i>S. neyagawaensis</i>	ATCC 27449 ^T		37	1,478	D63869
<i>S. sampsonii</i>	ATCC 19812 ^T		37	1,478	D63861
<i>S. scabies</i>	ATCC 49173 ^T		37	1,478	D63862
<i>S. setonii</i>	ATCC 25497 ^T		37	1,480	D63872
<i>S. tendae</i>	ATCC 19812 ^T		37	1,479	D63873
<i>S. turgidiscabies</i>	ATCC 700248 ^T		37	1,478	D63866
<i>K. azaticus</i>	IFO 13803		40	1,475	U93312
<i>K. brunnae</i>	IFO 14879		40	1,469 ^e	U93314
<i>K. griseola</i>	JCM 3339		R. K. Wilson ^f	1,468 ^e	N55221
<i>K. mediocidica</i>	IFO 14879		40	1,476	U93324
<i>K. setae</i>	JCM 3304		R. K. Wilson ^f	1,473	N55220

^a Isolated in Finland (21) or obtained from the type culture collections or the persons indicated. Five Finnish strains have been deposited to the Deutsche Sammlung für Mikroorganismen und Zellkulturen (DSMZ).

^b Assigned to these strain groups by Lindholm et al. (21) based on phenotypical and physiological data.

^c Length of 16S rRNA gene sequences excluding the first 29 nucleotides and the last 23 nucleotides of the 16S rRNA gene.

^d GenBank/EMBL/DBJ nucleotide sequence accession numbers.

^e Sequences lack nucleotides 1,135 to 1,141 (*Escherichia coli* numbering) (4).

^f Unpublished data.

8,000 × g for 2 min. The supernatant was transferred to a new tube, and the remaining cell debris was washed twice by resuspension in TENP buffer and centrifuged at 8,000 × g for 2 min. All the supernatants were finally combined, 7 µl of RNase A (10 µg/µl) was added, and the tube was incubated at 37°C for 30 min. RNase

A (and other protein contaminants) were removed by two subsequent phenol/chloroform extractions. DNA was precipitated by adding 0.6 volume of ice-cold isopropanol and centrifuged at 8,000 × g for 15 min. The supernatant was discarded and the pellet washed in 70% ethanol and then redissolved in 40 µl

Fig. 1. Sequence alignment of the major variable regions (δ , γ , α , β , and ϵ) of 16S rRNA genes of 10 *Streptomyces* strains isolated from potato scab lesions in Finland (bold), other strains of *Streptomyces* species reported to infect potato, and two *Kitosatospora* spp. Sequences are aligned with the sequence of *S. scabies* ATCC 49173^T, but the nucleotide numbers correspond to the 16S rRNA gene of *Escherichia coli* (4). The 16S rRNA gene of *E. coli* contains seven nucleotides that are missing in the δ region of the strains of *Streptomyces* and *Kitosatospora* species (indicated by dashes), whereas the eight nucleotides boxed in the β region are missing in *E. coli*. The positions of the variable regions are indicated in the drawing of the 16S rRNA gene above the sequence alignment. Only the nucleotides different from *S. scabies* ATCC 49173^T are shown, whereas identical nucleotides are indicated with dots. Deletions are indicated by dashes. The underlined sequences of strain 287 indicate the positions of primers specific to the Finnish strains of *S. turgidiscabies* (described in text).

of sterile distilled water. This procedure usually yielded 1 to 2 µg of DNA.

Polymerase chain reaction (PCR) amplification, sequencing, and phylogenetic analysis of 16S rRNA genes. The 16S rRNA-encoding sequence was amplified as two partially overlapping fragments using the previously described primer pairs pA-pE' and pA-pF' (9) for the 5' half and the primer pair pH'-pC (9) for the 3'

half of the gene. The reaction mix contained 50 pmol of each primer, 10 µmol of each deoxyribonucleoside triphosphate, 10 mM Tris-HCl (pH 8.0), 1.5 mM MgCl₂, 50 mM KCl, 0.1% Triton X-100, 1 unit of DyNAzymeII DNA polymerase (Finnzymes OY, Espoo, Finland), and 100 ng of total DNA. Sterile distilled water was added to a total volume of 100 µl of reaction mixture. PCR amplification was carried out with a thermal cycler (PTC-150 Mini-

TABLE 2. Similarity matrix of 16S rRNA sequences of *Streptomyces* strains isolated from potato scab lesions in Finland and some reference strains of *Streptomyces* spp. and *Kitosatospora* spp.

Strain, isolate, or species	<i>K. griseola</i>	<i>K. setae</i>	317	<i>S. aureofaciens</i>	380	SSC 101	289	302	267	<i>S. scabies</i> (ATCC 49173)	<i>S. diastatochromogenes</i>	364	298	287	300	<i>S. turgidiscabies</i>	<i>S. griseus</i> (2)	<i>S. griseus</i> (1)	<i>S. setonii</i>	<i>S. griseus</i> subsp. <i>griseus</i>	<i>S. acidiscabies</i>	' <i>S. griseus</i> subsp. <i>scabies</i> '	<i>S. tendae</i>	<i>S. lydicus</i>
<i>K. griseola</i>																								
<i>K. setae</i>	96.5																							
317	96.2	95.6																						
<i>S. aureofaciens</i>	96.1	95.4	99.8																					
380	93.8	94.4	95.8	95.8																				
SSC 101	93.8	94.4	95.8	95.8	100																			
289	93.8	94.4	95.8	95.8	100	100																		
302	93.8	94.4	95.8	95.8	100	100	100																	
267	93.8	94.4	95.8	95.8	100	100	100	100																
<i>S. scabies</i> (ATCC 49173)	94.0	94.4	95.9	95.9	99.9	99.9	99.9	99.9	99.9															
364	93.8	94.4	95.8	95.8	100	100	100	100	100	99.9														
<i>S. diastatochromogenes</i>	93.6	94.5	95.4	95.4	99.2	99.2	99.2	99.2	99.1	99.2														
298	94.3	94.6	96.9	96.9	97.7	97.7	97.7	97.7	97.7	97.6	97.4	97.7												
287	94.3	94.6	96.9	96.9	97.7	97.7	97.7	97.7	97.7	97.6	97.4	97.7	100											
300	94.3	94.6	96.9	96.9	97.7	97.7	97.7	97.7	97.7	97.6	97.4	97.7	100	100										
<i>S. turgidiscabies</i>	94.3	94.6	96.9	96.9	97.7	97.7	97.7	97.7	97.7	97.5	97.7	99.9	99.9	99.9										
<i>S. griseus</i> (2)	94.0	94.5	96.1	96.1	97.0	97.0	97.0	97.0	97.0	96.8	97.0	97.6	97.6	97.6	97.6									
<i>S. griseus</i> (1)	94.0	94.5	96.1	96.1	97.0	97.0	97.0	97.0	97.0	96.8	97.0	97.6	97.6	97.6	97.6	100								
<i>S. setonii</i>	94.1	94.5	96.3	96.2	97.2	97.2	97.2	97.2	97.2	97.1	97.2	97.6	97.6	97.6	97.7	99.9	99.9							
<i>S. griseus</i> subsp. <i>griseus</i>	94.0	94.5	96.1	96.0	97.0	97.0	97.0	97.0	97.0	97.1	96.9	97.0	97.6	97.6	97.6	100	100	99.9						
<i>S. acidiscabies</i>	93.8	95.1	95.8	95.7	97.5	97.5	97.5	97.5	97.5	97.5	97.5	97.5	97.2	97.2	97.2	97.3	97.2	97.2	97.4	97.3				
' <i>S. griseus</i> subsp. <i>scabies</i> '	92.7	93.0	94.9	94.9	95.7	95.7	95.7	95.7	95.7	96.0	95.7	96.6	96.6	96.6	96.7	95.6	95.6	95.5	95.6	96.6				
<i>S. tendae</i>	92.4	92.8	94.5	94.5	96.3	96.3	96.3	96.3	96.3	96.4	96.2	96.3	96.1	96.1	96.1	96.3	96.3	96.3	96.5	96.4	96.9	97.8		
<i>S. lydicus</i>	92.9	93.4	94.9	94.8	96.3	96.3	96.3	96.3	96.3	96.3	96.3	96.3	96.1	96.1	96.1	96.2	97.0	97.0	96.8	97.0	96.6	96.6	96.4

Fig. 2. Phylogenetic tree derived from 16S rRNA gene sequences of *Streptomyces* spp. and *Kitosatospora* spp. Scale indicates the Kimura nucleotide distance (K_{nuc}).

Cycler with Hot-bonnet; MJ Research, Watertown, MA) using the following program: initial denaturation for 3 min at 95°C, followed by 35 cycles consisting of denaturation for 30 s at 95°C, annealing for 30 s at 55°C, and DNA synthesis for 80 s at 72°C. A final extension of 3 min at 72°C was added at the end of the 35 cycles. The sequence of the amplified product was determined by direct sequencing of both strands using the solid phase method described previously (29). Sequencing reactions were analyzed using an automated DNA sequencer (ALFexpress; Pharmacia Biotechnology, Uppsala, Sweden).

PCR-amplified 16S rRNA genes of the nonpathogenic strains were not sequenced, but they were analyzed by restriction with endonucleases *AluI*, *MboI* (Fermentas, Vilnius, Lithuania), and *HaeIII* (New England Biolabs, Beverly, MA). In addition, strains previously placed in group 4 (21) were tested by PCR using species-specific primers 5'-CCTCGCATGGGGTGGGTTA-3' (16S rRNA gene nucleotides 195 to 216) (Fig. 1) and 5'-CGACAGCTCCCT-CCCCGTGA-3' (nucleotides 1,460 to 1,479), based on the 16S rRNA gene sequences of the Finnish strains 287, 298, and 300

(Fig. 1). Strain 317 was tested by PCR with primers 5'-CCGTC-GAAGGTGGGACCA-3' and 5'-TCCCTCAGGACCCAACAA-3', specific for *Kitosatospora* spp. (40).

Alignments and computer analyses of the DNA sequences were done using the Wisconsin Package software (version 8; Genetics Computer Group, Madison, WI). Sequences were aligned with the Pileup program. The alignments were used to calculate phylogenetic distances with the Distances program using the Kimura two-parameter model (17). A phylogenetic tree was compiled with the neighbor-joining method (30) using the Growtree program. Significance of the branch order was tested by 1,000 replications of bootstrap sampling of the original nucleotide sequence alignments by the Treecon program (38).

PCR amplification and restriction analysis of the *necl* gene and the ORF*mp*-*necl* intergenic region. A previous study suggested that the *necl* gene of *S. scabies* can be used as a marker for pathogenicity in *Streptomyces* spp. (5). ORF*mp* is a transposase pseudogene located upstream and separated by an intergenic region from *necl*. Sequences of these three regions form a genetic element that is reported to be identical in pathogenic *S. scabies* and *S. turgidiscabies* strains (5). *necl* and the intergenic region between ORF*mp* and *necl* were amplified using the previously published PCR primers Nf-Nr and If-Ir, respectively. Primers If and Ir are targeted to an internal sequence of ORF*mp* and *necl*, respectively (5). Amplified fragments were analyzed by restriction with endonucleases *AluI*, *MboI*, and *HaeIII*.

Pathogenicity tests. Strains 364 (*S. scabies*); 287, 298, and 300 (group 4); and 317 were grown in oatmeal broth (21) at 28°C with shaking (150 rpm) for 10 days. After growth, the color of the broth was different for strain 364 (yellowish); 287, 298, and 300 (light brown); and 317 (cream). The cultures were diluted 1:1 with sterile distilled water, and 250 ml was poured into a pot (20-cm diameter) filled with heat-sterilized greenhouse compost. Inoculum density of cultures was checked by dilution-plating on PDA and counting colonies (approximately 11×10^6 CFU per 250 ml). Pathogen-free tubers of 'Bintje' and 'Matilda' (susceptible to netted scab and common scab) (2,21,31) were obtained from the Seed Potato Center, Tynärvä, Finland, and sprouted in dim light in the greenhouse for 2 weeks. One tuber was placed into each pot immediately after the inoculum had been mixed into the compost. Two pots of each cultivar were inoculated with each *Streptomyces* strain, and four pots of each cultivar were not inoculated (negative controls). Plants were grown in the greenhouse during January to May under natural daylight supplemented with illumination from fluorescent lamps (temperature 20 to 22°C). Reisolation of inoculated bacteria from symptomatic tubers was carried out as previously described (21).

RESULTS

Analysis of 16S rRNA genes. The sequence corresponding to 16S rRNA nucleotide positions 29 to 1,521 (according to the nomenclature of *Escherichia coli*) (4) was determined for 10 *Streptomyces* strains isolated from scab lesions on potato tubers in Finland (21). It was also determined for five additional *Streptomyces* strains isolated elsewhere that represented three species causing scab on potato. These sequences and the previously published 16S rRNA gene sequences of other *Streptomyces* spp. (16,37) reported to cause scab on potato were aligned (Fig. 1) with the corresponding sequence of *S. scabies* ATCC 49173^T (37). The highly variable γ region (nucleotides 181 to 213) and the less variable regions α (nucleotides 999 to 1,025) and β (nucleotides 1,133 to 1,143) previously reported by Stackebrandt et al. (35) were detected (Fig. 1). Two additional regions designated δ (nucleotides 71 to 102) and ϵ (nucleotides 1,446 to 1,464) also contained considerable variation (Fig. 1) not previously reported. When the entire sequenced region is taken into consideration, sequence identities (SI) among the *Streptomyces* spp. analyzed ranged from 94.5 to 100% (Table 2).

Fig. 3. A, Symptoms on tubers of potato 'Bintje' caused by strains 287 (*Streptomyces turgidiscabies*) (left) and 364 (*S. scabies*) (center). A tuber grown in noninoculated soil is to the right. B, Severe symptoms caused by strain 298 (*S. turgidiscabies*) in potato 'Matilda'. C, Netted scab on the tuber and a necrotic lesion on a stolon of 'Bintje' caused by strain 317 (*S. aureofaciens*). Tubers in photographs appear approximately half of their natural size.

However, the lengths of the 16S rRNA gene sequences varied among strains because of deletions or insertions (Table 1, Fig. 1).

Based on SI and phylogenetic analyses, the Finnish strains were placed into three groups (Fig. 2). Two of these groups consisted of strains with almost identical 16S rRNA sequences (99.9% SI) within the group but differing by at least 0.8% from the strains in other groups. The first group included most of the Finnish strains (267, 289, 302, 364, 380, and SSC 101), *S. scabiei* ATCC 49173^T, and the two other *S. scabiei* strains, ATCC 33282 and SNS-26, analyzed by Takeuchi et al. (37). The Finnish strains were identical but differed from the latter-mentioned *S. scabiei* strains at nucleotide positions 185 (G→A) and 1,446 (T→C) and by a deletion of the nucleotide at position 1,458 (99.9% SI) (Fig. 1, Table 2). The plant-pathogenic species *S. diastatochromogenes* ATCC 12309^T was linked to this cluster (99.1% SI to *S. scabiei* ATCC 49173^T).

The second group consisted of the Finnish strains 287, 298, and 300 (previously placed in group 4) (21) and the Japanese strain *S. turgidiscabiei* ATCC 700248^T. Finnish strains were identical but differed from the Japanese strain of *S. turgidiscabiei* at nucleotide positions 212 (C→A) and 1,454 (C→T) and the presence of nucleotide 1,449 (C) missing in the Japanese strain.

The Finnish strain 317 and *S. aureofaciens* NRRL 2209^T differed from each other at three nucleotide positions (76 [A→G], 77 [G→C], and 1,475 [T→C]) and grouped with *Kitosatospora* spp., forming a distinct cluster. The two strains of *S. aureofaciens*, *K. brunnae* IFO 14879 and *K. griseola* JCM 3339 (Table 1), lacked seven nucleotides (nucleotides 1,135 to 1,141). This gap was unique for these strains and existed in neither *S. scabiei* ATCC 49173^T nor any other strain analyzed in this study (Fig. 1). Furthermore, amplification of the 16S rRNA gene with the *Kitosatospora*-specific primers of Zhang et al. (40) resulted in amplification of a product from *S. aureofaciens* but not from *S. scabiei* and *S. turgidiscabiei* (data not shown).

16S rRNA sequences of the two strains of *S. griseus* included in this study were identical but contained two additional nucleotides (1,137 [C] and 1,263 [C]) as compared with *S. griseus* subsp. *griseus* KCTC 9080 (16). Comparison of the two *S. griseus* strains to *S. setonii* ATCC 25497^T (37) revealed two nucleotide changes (87 [T→C] and 465 [G→A]) and two additional nucleotides (79 [G] and 93 [T]) in *S. setonii*. These four strains formed their own cluster.

The 16S rRNA gene sequence of '*S. griseus* subsp. *scabiei*' ATCC 10246 was identical to that of *S. sampsonii* ATCC 19812^T (37). Their branch in the phylogenetic tree was linked to a larger branch of less-related species, of which *S. lydicus* contains plant-pathogenic strains (1).

Symptoms in tubers. Strains 364 (*S. scabiei*); 287, 298, and 300 (group 4); and 317 were tested for pathogenicity on 'Bintje' and 'Matilda'. All strains were pathogenic and caused symptoms, whereas tubers grown in noninoculated soil remained symptomless (Fig. 3). Strain 364 of *S. scabiei* caused characteristic common scab symptoms (Fig. 3). Symptoms caused by strains 287, 298, and 300 of *S. turgidiscabiei* were identical. Lesions were black and pitted on 'Bintje' and pitted or superficial on 'Matilda' (Fig. 3). Symptoms caused by these three strains were clearly distinguishable from those caused by strain 364 in 'Bintje' (Fig. 3) but were more similar to those caused by strain 364 in 'Matilda'. Strain 317 of *S. aureofaciens* was not severe and caused only superficial necrosis resembling netted scab. Necrotic lesions developed on stolons of both cultivars (Fig. 3). This was not observed with strains 364, 287, 298, and 300.

PCR amplification and restriction analysis of the *necl* gene and the ORF_{tnp}-*necl* intergenic region. *necl* and the intergenic region between *necl* and its 5' transposase pseudogene ORF_{tnp} are highly conserved and linked with thaxtomin production and pathogenicity in *S. scabiei* and *S. turgidiscabiei*, according to a previous study (5). *necl* and the intergenic region were successfully amplified by PCR from ATCC 49173^T and pathogenic Finnish strains of *S. scabiei* (including strains 364 and 380) using the

originally described primers (5) (Fig. 4). Restriction analysis of the products revealed the expected pattern of fragments (5,6), which was identical in all strains. In contrast, no product was obtained with the two pairs of primers from the nonpathogenic strain 346 of *S. scabiei*; pathogenic strains 287, 298, and 300; and the nonpathogenic strains 268 and 283 of *S. turgidiscabiei* and strain 317 of *S. aureofaciens* (Fig. 4).

DISCUSSION

Grouping of the 10 pathogenic Finnish *Streptomyces* strains based on phylogenetic analysis of 16S rRNA gene sequences was supported by the previous grouping based on morphological and physiological traits (21) and indicated that the strains belonged to three previously described, plant-pathogenic species: *S. scabiei* (strains SSC 101, 267, 289, 302, 364, and 380), *S. turgidiscabiei* (strains 287, 298, and 300), and *S. aureofaciens* (strain 317). Most of the sequence variability was confined to five regions, namely the previously reported α , β , and γ regions (24) and the new δ and ϵ regions detected in this study. The γ region was the most variable of these regions. Our phylogenetic analysis was fully consistent with the previous study by Takeuchi et al. (37), which included fewer species and strains. Important new findings of this study included the 16S rRNA gene sequence-based phylogenetic positions of *S. lydicus*, the previously described strains and subspecies of *S. griseus*, and the placement of *S. aureofaciens* to the distinct phylogenetic cluster of *Kitosatospora* spp.

S. scabiei is the most studied *Streptomyces* species infecting potato and is reported to show exceptionally broad intraspecific genetic diversity (14,27). This study indicated that 16S rRNA genes of *S. scabiei* possess some, even though limited, intraspecific variability. The previous study of Takeuchi et al. (37) found

Fig. 4. Polymerase chain reaction amplification of the **A**, 16S rRNA gene using universal primers (9); **B**, 16S rRNA gene using the primers specific to Finnish strains of *Streptomyces turgidiscabiei* designed in this study; **C**, *necl* gene; and **D**, intergenic region between ORF_{tnp} and *necl*. P = pathogenic on potato; NP = nonpathogenic; λHE = molecular marker (bacteriophage λ DNA digested with *Hind*III and *Eco*RI; sizes in base pairs are indicated for selected fragments); 53 = *S. scabiei* ATCC 49173^T; 346, 364, and 380 = Finnish strains of *S. scabiei*; 268, 283, 287, 298, and 300 = Finnish strains of *S. turgidiscabiei*; and 317 = Finnish strain of *S. aureofaciens*.

that 16S rRNA gene sequences in three strains of *S. scabies* with geographically different origins (Japan, Hungary, and United States) are identical except for one nucleotide position. While the 16S rRNA genes of the six Finnish strains of *S. scabies* analyzed in this study were all identical, they differed from the three previously analyzed strains of *S. scabies* (37) at one or two nucleotide positions and had one additional nucleotide (99.9% SI). Secondly, this study helped to clarify the taxonomic status of certain strains that have been previously considered to be deviant strains of *S. scabies*, based on morphological and physiological characterization. Such strains in previous studies included those not producing melanin pigment, not utilizing one of the ISP sugars, and lacking flexuous sporophores on YME, but possessing all other traits of *S. scabies* (10,13,21). The 16S rRNA gene sequence of the Japanese, deviant, non-melanin-producing strain SNS-26 of *S. scabies* is identical to the 16S rRNA gene sequence of one melanin-producing strain (ATCC 3328) and differs by one nucleotide from *S. scabies* ATCC 49173^T (37). Similarly, 16S rRNA gene sequences of Finnish strain 364, which does not utilize D-xylose, and strain 380, which does not produce spiral sporophores on YME, were identical to the other four Finnish strains of *S. scabies*. The latter four strains, in turn, are identical to *S. scabies* ATCC 49173^T for all tested physiological and morphological traits, except for some differences in their whole-cell fatty acid profiles (21). The characteristic common scab symptoms caused by strain 364 in 'Bintje' and 'Matilda' provided further evidence that this physiologically deviant strain belongs to *S. scabies*.

Strain 317 was identified as *S. aureofaciens*, because this strain and *S. aureofaciens* NRRL 2209^T are phenotypically identical (12, 21), and they were placed in the same phylogenetic cluster based on 16S rRNA gene sequences (99.8% SI). The symptoms caused by strain 317 resembled netted scab or russet scab, both of which are similar (31), which also supports assignment of strain 317 to *S. aureofaciens*, reported to cause russet scab in North America (12,23,31). Strain 317 was originally isolated from one farm in Loimaa, and similar strains have not been isolated elsewhere in Finland (21). To our knowledge, this is the first time *S. aureofaciens* has been reported in Europe.

S. aureofaciens was placed in the minor cluster group 14 (*S. aureofaciens*) by Williams et al. (39) and in subcluster 22-4 (*S. misakiensis*) by Kämpfer et al. (15). Both of the strains of *S. aureofaciens* in this study carried a 7-base pair (bp) deletion within the 16S rRNA gene, which has not been reported in any *Streptomyces* spp. but exists in two of the five *Kitosatospora* spp. included in this study (Fig. 1). Phylogenetic analysis of 16S rRNA genes placed *S. aureofaciens* and *Kitosatospora* spp. in the same distinct cluster (irrespective of the 7-bp deletion), and the *Kitosatospora*-specific primers (40) detected the 16S rRNA gene of only *S. aureofaciens*. When the 16S rRNA gene sequence of *E. coli* (4) was included to root the phylogenetic tree, the phylogenetic branch formed by *Kitosatospora* spp. and *S. aureofaciens* became even more distinct (data not shown). These data were consistent with the phylogenetic analysis of Zhang et al. (40), which placed several other *Streptomyces* spp. in the same cluster with *Kitosatospora* spp. Our data indicate that *S. aureofaciens* may be a member of the recently revived genus *Kitosatospora* (26,40).

S. turgidiscabies was recently found on the eastern side of Hokkaido island in Japan (37) and it has not been reported elsewhere (25). However, morphologically and physiologically similar pathogenic strains occur throughout a broad area in Finland (21). The 16S rRNA gene sequences of three Finnish strains were identical and differed from Japanese strain *S. turgidiscabies* ATCC 700248^T (91-Sy-13) (37) at only three nucleotide positions (99.9% SI). Thus, all four strains seem to belong to the same species. To our knowledge, this is the first study reporting the occurrence of *S. turgidiscabies* outside Japan. A recent study suggested that Japanese strains of *S. turgidiscabies* gained their pathogenicity factors from *S. scabies* (5). This conclusion was based on the presence of an iden-

tical, virulence-associated genetic element in pathogenic strains of both species (data on nonpathogenic strains of the two species not shown). The genetic element consists of a transposase pseudogene (ORF_{tnp}), a gene (*nec1*) that allows nonpathogenic *S. lividans* to induce necrosis on potato tuber disks, and an intergenic region between these two genes. *nec1* has been found in 23 and 15 pathogenic strains of *S. scabies* and *S. turgidiscabies*, respectively, except for one pathogenic strain of *S. scabies* from South Africa. Therefore, it has been proposed that PCR amplification of *nec1* could be used as a marker for pathogenicity in *Streptomyces* spp. and might substitute for pathogenicity testing (5). Our data were consistent with this view as regards *S. scabies*. In contrast, neither *nec1* nor the intergenic region could be amplified from the pathogenic Finnish strains of *S. turgidiscabies* and *S. aureofaciens*, which indicated that either the genetic element is not present or its sequence is not conserved in these strains. This needs to be resolved in further studies.

The pathogenicity of all Finnish *Streptomyces* strains of this study has been previously tested repeatedly (21) with the minituber method (20) as described by Loria et al. (22). Japanese strains of *S. turgidiscabies* have also been tested for pathogenicity with this method (5). Data on Japanese and Finnish strains of *S. turgidiscabies* are consistent in that *S. turgidiscabies* induces necrosis on potato minitubers (5,21). The original report of *S. turgidiscabies* states that "scab symptoms caused by this pathogen (represented by strain 91-Sy-13) are indistinguishable from those caused by *S. scabies*" in mature potato tubers (37). However, in a more recent report, the symptoms caused by *S. turgidiscabies* in experimentally inoculated potatoes have been described as "erumpent, rough, corky lesions" and the symptoms on tubers in the field were described as "distinctly erumpent" (25). In our study, the Finnish strains of *S. turgidiscabies* caused mainly pitted lesions that were distinct from the symptoms caused by *S. scabies* and *S. aureofaciens* (Fig. 3). Discrepancies in the types of symptoms reported for *S. turgidiscabies* may be attributable to the different potato cultivars used in each study. For example, *S. scabies* is known to cause different symptoms (erumpent, superficial, or pitted lesions) depending on virulence of the pathogen strain and susceptibility of the potato cultivar (23). Alternatively, the different symptoms caused by the Finnish and Japanese strains and the possible absence of the ORF_{tnp-nec1} region in the Finnish strains suggests that they may use somewhat different pathogenicity factors, which remains to be investigated in future studies.

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