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Phylogenetic analysis of *Streptomyces* spp. isolated from potato scab lesions in Korea on the basis of 16S rRNA gene and 16S–23S rDNA internally transcribed spacer sequences

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The 16S rRNA gene sequences for 34 strains, including 11 isolates, were determined to classify scab-causing *Streptomyces* spp. and relatives isolated from potato scab lesions collected in Jeju, Korea. The 16S–23S rDNA internally transcribed spacer (ITS) sequences were determined to investigate whether the 16S–23S ITS region is useful for analysing intra- and interspecific relationships in these bacteria. On the basis of phylogenetic analysis of 16S rRNA gene sequences, most of the isolates were classified as *Streptomyces scabiei* and *Streptomyces acidiscabies*. Isolate KJO61 was placed in an ambiguous taxonomic position between *Streptomyces reticuliscabiei* and *Streptomyces turgidiscabies*. 16S–23S ITS region sequence analysis showed that tRNA genes were not found in this region of *Streptomyces* spp. The 16S–23S ITS regions of *Streptomyces* spp. exhibited various lengths and highly variable sequence similarities (35–100 %) within strains as well as intra- and interspecies. It was revealed that *Streptomyces europaeiscabiei* could be clearly differentiated from *Streptomyces scabiei*. However, it was clarified that ITS regions are not useful in phylogenetic analysis of *Streptomyces* spp.

INTRODUCTION

Streptomyces species are abundant micro-organisms in soil and are well known for their ability to produce biologically active secondary metabolites, particularly antibiotics. Interestingly, only a small number of *Streptomyces* species are known to be plant or animal pathogens (Loria *et al.*, 1997). Plant-pathogenic *Streptomyces* species cause diseases of diverse root crops such as potato, radish, turnip, beet, carrot and sweet potato (Labeda & Lyons, 1992; Goyer & Beaulieu, 1997). Scab diseases of potatoes and other root crops, except sweet potato, are characterized by corky lesions which may appear as shallow, raised or deep pitted lesions on potato tubers and expanded tap roots and cause economically significant losses in yield (Loria *et al.*, 1997). These diseases are known to be caused by *Streptomyces scabiei* (Lambert & Loria, 1989a; Trüper & Clari, 1997), *Streptomyces acidiscabies* (Lambert & Loria, 1989b), *Streptomyces turgidiscabies* (Miyajima *et al.*, 1998) and other species (Faucher *et al.*, 1995; Goyer *et al.*, 1996; Bouchek-Mechiche *et al.*, 2000). *Streptomyces scabiei* is the predominant causal agent and most closely related to the Diastatochromogenes group encompassing *Streptomyces diastatochromogenes*, *Streptomyces bottropensis* and *Streptomyces neyagawaensis* (Takeuchi *et al.*, 1996). *Streptomyces acidiscabies* and other pathogenic strains fall into a group with similarities to the *Streptomyces albidoflavus* group of Williams *et al.* (1983).

The taxonomy of potato-scab-causing *Streptomyces* spp. has been studied by many microbiologists on the bases of numerical analysis of phenotypic characteristics (Faucher *et al.*, 1995), fatty acid and protein profile analyses (Paradis *et al.*, 1994), DNA–DNA hybridization (Healy & Lambert, 1991; Bouchek-Mechiche *et al.*, 2000) and 16S rRNA gene sequence analysis (Takeuchi *et al.*, 1996; Kreuze *et al.*,

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Abbreviation: ITS, internally transcribed spacer.

A neighbour-joining tree of isolates and *Streptomyces* spp. related to potato scab disease on the basis of 16S–23S ITS rDNA sequences is available in IJSEM Online.

1999). 16S rRNA gene sequencing is a powerful method for elucidating phylogenetic relationships among prokaryotic organisms (Woese, 1987; Stackebrandt *et al.*, 1997) and has been used to facilitate the differential identification of the genus *Streptomyces* (Mehling *et al.*, 1995; Kreuze *et al.*, 1999). Nevertheless, 16S rRNA gene sequences may be insufficient to define phylogenetic relationships among closely related species and among strains belonging to a species because of the evolutionary conservation of 16S rRNA (Woese, 1987). In comparison with 16S rRNA gene sequences, sequences of 16S–23S rDNA internally transcribed spacer (ITS) regions are more variable and have been shown to be useful in inferring the phylogenetic relationships between closely related organisms (Gürtler & Stanisich, 1996). Recently, the number, size and sequences of 16S–23S ITS regions were used for discrimination of *Streptomyces albidoflavus* strains (Hain *et al.*, 1997), genetic analysis of the genus *Nocardioides* (Yoon *et al.*, 1998) and clarification of the relationship between members of the family *Thermomonosporaceae* (Zhang *et al.*, 2001).

In this study, we determined 16S rRNA gene sequences to classify representative scab-causing *Streptomyces* spp. and isolates from potato scab lesions collected in Jeju, Korea, and sequenced the 16S–23S ITS region to investigate whether this is useful for the analysis of intra- and inter-specific relationships between scab-causing *Streptomyces* spp. and isolates.

METHODS

Bacterial strains and culture conditions. The strains used in this study are listed in Table 1. The strains from Jeju, Korea, were isolated from scab lesions of field-grown potato tubers from different geographic locations. All strains used in this study were grown in shaking flasks containing GYM (*Streptomyces* medium; DSM Z Medium 65) at 28 °C. All strains were stored at −70 °C as suspensions of spores and hyphae in 15 % (v/v) glycerol.

Isolation of chromosomal DNA. Chromosomal DNAs were isolated by a versatile quick-prep method for genomic DNA of Gram-positive bacteria (Pospiech & Neumann, 1995), with some modifications. Mycelia (1–2 ml) grown in a GYM broth shake culture were centrifuged, rinsed with TE and resuspended in 0·4 ml SET buffer (75 mM NaCl, 25 mM EDTA, 20 mM Tris, pH 7·5). Lysozyme was added to a concentration of 1 mg ml^{-1} and incubated at 37 °C for 0·5–1 h. Then 0·1 vols 10 % SDS and 0·5 mg Proteinase K ml^{-1} were added and incubated at 55 °C with occasional inversion for 2 h. One-third volume 5 M NaCl and 1 vol. chloroform were added and incubated at room temperature for 0·5 h with frequent inversion. The mixture was centrifuged at 4500 ***g*** for 15 min and the aqueous phase was transferred to a new tube using a blunt-ended pipette tip. Chromosomal DNA was precipitated by the addition of 1 vol. 2-propanol with gentle inversion. The DNA was transferred to a new tube, rinsed with 70 % ethanol, dried under vacuum and dissolved in a suitable volume (about 100 µl) of distilled water. The dissolved DNA was treated with 20 µg RNase A ml^{-1} at 37 °C for 1 h. Samples were extracted in the same volume of phenol/chloroform/isoamyl alcohol (25 : 24 : 1) and precipitated with 2·5 vols cold ethanol and 0·1 vols 3 M sodium acetate. The pellets were washed with 70 % ethanol, dried and dissolved in TE or distilled water.

PCR amplification, cloning and sequencing of 16S rRNA genes and 16S–23S ITS regions. The 16S rRNA gene was amplified using primers fD1 (5′-AGAGTTTGATCCTGGCTCAG-3′) and rP2 (5′-ACGGCTACCTTGTTACGACTT-3′) (Weisburg *et al.*, 1991). The primers for amplification of the DNA fragment containing the 16S–23S ITS region were designed in this study. The sequences of the oligonucleotide primers annealing to the 16S rRNA, 16S–23S ITS and 23S rRNA flanking regions were 5′-GTCAAGTCATCATGCCCCTT-3′ [primer ITSL, positions 1176–1195 (*Streptomyces scabiei* 16S rRNA numbering; GenBank no. AB026199)] and 5′-AAACTTGGCCACAGATGCTC-3′ [primer ITSR, positions 1846–1828 (*Streptomyces scabiei* 16S–23S ITS and 23S rRNA numbering; GenBank no. AB026199)], respectively. The 16S rRNA gene was sequenced with primers designed by Chun & Goodfellow (1995) and the 16S–23S ITS region was sequenced by a primer (16S rRNA region 3, 5′-AAGTCGTAACAAGGTA-3′) designed by Weisburg *et al.* (1991).

Amplification of the 16S rRNA and 16S–23S ITS regions was performed in a Peltier Thermal Cycler PTC 200 (MJ Research) in a total volume of 50 µl containing 30–50 ng DNA, 100 µM each primer, 10 µM dNTP, 10× buffer (100 mM Tris/HCl, pH 8·0, 500 mM KCl, 20 mM $MgCl_2$, 0·1 % gelatin) and 1·5 U *Taq* DNA polymerase (Promega). PCR was performed under the following conditions: 4 min at 94 °C, followed by 35 cycles of 1 min at 94 °C, 1 min at 58 °C, 2 min at 72 °C. Cloning and sequencing of 16S rRNA and the 16S–23S ITS region have been described previously (Song *et al.*, 2001).

Selection of multiple alleles of the 16S–23S ITS region. Multiple alleles were selected on the basis of RFLP with restriction enzymes *Hae*III and *Msp*I or by random alignment with sequences of 30 clones per strain.

Data analysis. The sequences of 16S rRNA and 16S–23S ITS determined in this study were aligned using CLUSTAL W software (Thompson *et al.*, 1994). The nucleotide similarity values were calculated from the alignment. Evolutionary distance matrices were constructed using the algorithm of Jukes & Cantor (1969) and evolutionary trees for the datasets were inferred from the neighbour-joining method (Saitou & Nei, 1987) using MEGA version 2.1 (Kumar *et al.*, 2001). The stability of relationships was assessed by performing bootstrap analysis of neighbour-joining data based on 1,000 resamplings.

RESULTS AND DISCUSSION

Analysis of 16S rRNA gene sequence

16S rRNA gene sequence analysis was carried out to elucidate the taxonomic position of isolates from Jeju, Korea, with most of the known potato-scab-causing *Streptomyces* spp. We used the *Streptomyces scabiei* 16S rRNA gene sequence (GenBank no. AB026199) as a standard for sequence numbering to reduce the inconvenience of sequence analysis by gap penalties. 16S rRNA gene sequences (1424–1428 nt between positions 73 and 1498) were determined for 33 strains, including 11 isolates from Jeju, Korea, and were compared with the corresponding sequences of 17 type strains (Table 1) of representative scab-causing and related *Streptomyces* spp. The result of our phylogenetic analysis was consistent with previous studies by Takeuchi *et al.* (1996) and Kreuze *et al.* (1999), but slightly different from that of Bouchek-Mechiche

Table 1. The length of 16S–23S ITS regions, and intrastrain and intraspecies sequence similarities of isolates and scab-causing and related *Streptomyces* spp. used in this study

Streptomyces species	Strain (no. of alleles*)	KACC no.†	Length (bp)	Similarity (%)		Source and origin	Pathogenicity‡
				Intrastrain	Intraspecies		
S. scabiei	ASO2 (5)	20200	270–274	82·2–99·3	46·3–100	*Solanum tuberosum*, Korea	NC
	HJA3	20193	274			*Solanum tuberosum*, Korea	NC
	SSA4 (5)	20194	269–282	75·5–99·3		*Solanum tuberosum*, Korea	NC
	DBB1 (2)	20198	282	98·9		*Solanum tuberosum*, Korea	NC
	ADA1 (2)	20192	282	94·3–100		*Solanum tuberosum*, Korea	NC
	KCTC 1150	20050	274			Unknown	
	DSM 40962	20227	270			*Solanum tuberosum*, USA	
	DSM 41658^{T} (6)	20101	272–274	93·1–99·6		*Solanum tuberosum*, USA	+
	DSM 40778	20135	274			*Solanum tuberosum*, USA	
	DSM 41660	20222	274			*Solanum tuberosum*, USA	+
	DSM 40995	20136	278			*Solanum tuberosum*, USA	
	DSM 41659	20139	278			*Solanum tuberosum*, USA	+
	DSM 40961	20221	279			*Solanum tuberosum*, USA	
	DSM 40611	20134	262			USA	Weak
	DSM 41114	20138	270			USA	
	DSM 41005	20137	261			USA	Weak
S. europaeiscabiei	CFBP 4497^{T} (5)	20186	278–279	94·2–99·6	92·8–100	*Solanum tuberosum*, France	+
S. bottropensis	DSM 40262^{T} (5)	20131	273–285	78·8–99·6		Soil	
S. stelliscabiei	CFBP 4521^{T} (6)	20187	280–285	81·1–99·7		*Solanum tuberosum*, France	+
S. neyagawaensis	KCCM 12304^{T} (6)	20130	269–280	84·5–98·9		Soil	
S. ipomoeae	DSM 40383	20241	270			*Ipomoea batatas*, USA	+
S. diastatochromogenes	DSM 40449^{T} (6)	20133	258–268	81–99·2			
S. acidiscabies	KJA2	20190	245		74·4–99·2	*Solanum tuberosum*, Korea	NC
	ASG2		246			*Solanum tuberosum*, Korea	NC
	ASG8	20213	245			*Solanum tuberosum*, Korea	NC
	DSM 41671	20219	247			*Solanum tuberosum*, USA	+
	DSM 41670	20220	246			*Solanum tuberosum*, USA	+
	ADB1	20214	266			*Solanum tuberosum*, Korea	NC
	DSG1	20191	267			*Solanum tuberosum*, Korea	NC
	ATCC 49003^{T} (6)	20125	245–266	75·6–99·2		*Solanum tuberosum*, USA	+
	DSM 41669	20140	264			*Solanum tuberosum*, USA	+
S. reticuliscabiei	CFBP 4531^{T} (3)	20185	280–282	89–96·4		*Solanum tuberosum*, France	+
Streptomyces sp.	KJO61 (3)	20201	277–278	92·8–98·2		*Solanum tuberosum*, Korea	
Streptomyces sp.	DSM 41747	20240	281		91·8–97·9	*Solanum tuberosum*, Finland	+
	DSM 41745	20238	280			*Solanum tuberosum*, Finland	+
	DSM 41746	20239	282			*Solanum tuberosum*, Finland	+
S. turgidiscabies	ATCC 700248^{T} (4)	20121	281–286	85·4–96·8		*Solanum tuberosum*, Japan	+

Table 1. cont.

Streptomyces species	Strain (no. of alleles*)	KACC no.†	Length (bp)	Similarity (%)		Source and origin	Pathogenicity‡
				Intrastrain	Intraspecies		
S. griseus	ATCC 10137 (3)	20084	263–267	73·8–93·9			
S. setonii	ATCC 25497^T		260			*Solanum tuberosum*	Weak
S. griseofuscus	IFO 12870^T (3)	20083	247–280	78·8–91·2			
S. albidoflavus	DSM 40455^T (2)		276–284	85·1	78·6–98·2		
	DSM 40792 (2)		276–284	79·3			
S. sampsonii	DSM 40394^T (6)	20132	275–287	85·5–98·6		*Solanum tuberosum*	
S. eurythermus	KCCM 12267^T (8)	20129	261–272	73·7–99·6		Soil, Angola	
S. tendae	ATCC 19812^T		245			Soil, France	
S. aureofaciens	DSM 40127^T (6)	20180	236–241	89·4–99·2		Soil	

*The number of alleles in parentheses is the number of multiple copies of the 16S–23S ITS regions used in this study. ATCC, American Type Culture Collection, Manssas, VA, USA; CFBP, Collection Française des Bacteries Phytopathogenes, INRA, Beaucouzé, France; DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany; IFO, Institute for Fermentation, Culture Collection of Micro-organisms, Osaka, Japan; KCCM, Korean Culture Centre of Micro-organisms, Seoul, Republic of Korea; KCTC, Korean Collection for Type Cultures, Yusong, Taejon, Republic of Korea.

†KACC, Korean Agricultural Culture Collection, Suwon, Republic of Korea (http://kacc.rda.go.kr).

‡Pathogenicity data obtained from Bouchek-Mechiche *et al.* (2000), Lambert & Loria (1989a, b), Miyajima *et al.* (1998), Labeda & Lyons (1992) and the DSM catalogue. NC, Not confirmed.

et al. (2000) in which *Streptomyces turgidiscabies* was not included. Based on phylogenetic analysis (Fig. 1), cluster I, the Diastatochromogenes group, formed diverse phyletic lines, including *Streptomyces scabiei*, *Streptomyces europaeiscabiei*, *Streptomyces stelliscabiei*, *Streptomyces ipomoeae* and *Streptomyces diastatochromogenes*, supported by 87 % of bootstrap replicates. Three strains, DSM 40995, 40961 and 41659, and five isolates, ADA1, ASO2, DBB1, HJA3 and SSA4, were highly related to *Streptomyces europaeiscabiei* (genomospecies, Bouchek-Mechiche *et al.*, 2000), and *Streptomyces scabiei*. *Streptomyces stelliscabiei* and *Streptomyces ipomoeae* were related to *Streptomyces bottropensis* and *Streptomyces neyagawaensis* (98·4 and 98·6 %, respectively). The Scabiei group, encompassing *Streptomyces scabiei* and *Streptomyces europaeiscabiei*, exhibited very high similarity (>99 %) and distinct sequences (CTTGTG→GTAA-G, 1435–1439) from other groups as found by Kreuze *et al.* (1999). *Streptomyces europaeiscabiei* was differentiated from *Streptomyces scabiei* by one nucleotide substitution (G→A, 176) and therefore it is suggested that strains DSM 40995, 40961 and 41659 should be reclassified as *Streptomyces europaeiscabiei*. A DNA–DNA homology study and genetic characterization other than 16S rDNA analysis should be performed to confirm this opinion.

In cluster II, isolates ADB1, ASG2, ASG8, DSG1 and KJA2 were highly similar to *Streptomyces acidiscabies* (99·4–100 %), supported by 100 % of bootstrap replicates. *Streptomyces acidiscabies* had two distinct regions and a unique nucleotide for identification. The two distinct regions were CG→TC (position 175–176) and GATGAGTG→GGGCGGGGG (193–200). The unique nucleotide (A→G) was at position 376. The two distinct regions are positioned in a major variable region (γ-region) of 16S rRNA genes (Kreuze *et al.*, 1999). It is thought that the γ-region is a valid region for use in rapid identification of *Streptomyces* spp.

In cluster III, isolate KJO61 was similar to *Streptomyces reticuliscabiei* and *Streptomyces turgidiscabies* (99·4 and 99·6 %, respectively). *Streptomyces* strains DSM 41745, 41746 and 41747 were similar to *Streptomyces turgidiscabies* (99·5, 99·4 and 99·4 %) and *Streptomyces reticuliscabiei* (99·7, 99·6 and 99·7 %). This result was similar to an earlier study (Kreuze *et al.*, 1999), but isolate KJO61 and *Streptomyces reticuliscabiei* (CFBP 4531) were closer to *Streptomyces turgidiscabies* rather than Finnish strains DSM 41745, 41746 and 41747. It is considered that KJO61, *Streptomyces reticuliscabiei* and the Finnish strains should be subjected to further genetic analyses (especially DNA relatedness with ATCC 700248) to pinpoint their taxonomic position.

Cluster IV formed two phyletic lines, including diverse *Streptomyces* spp., supported by 100 and 99 % of bootstrap replicates, respectively. One included *Streptomyces griseus* and *Streptomyces setonii* and the other included *Streptomyces griseofuscus*, *Streptomyces albidoflavus*, *Streptomyces sampsonii*, *Streptomyces eurythermus* and *Streptomyces*

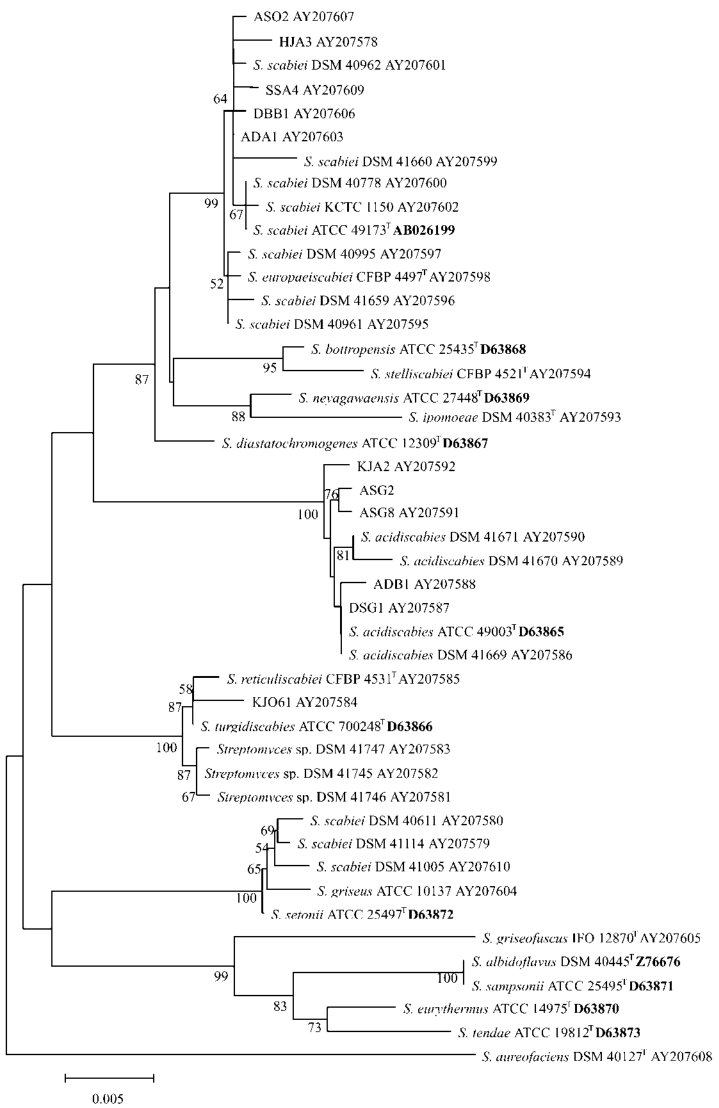

Fig. 1. Phylogenetic tree of 45 strains of scab-causing and related *Streptomyces* spp. based on the 16S rRNA gene sequence. Sequences of 11 type strains (in bold) were obtained from the GenBank. The numbers at the branching points are the percentages of occurrence in 1000 bootstrapped trees. The bar indicates a distance of 0·005 substitutions per site. The pathogenicity for potato of the Korean isolates was not confirmed.

tendae. *Streptomyces scabiei* DSM 40611, 41005 and 41114 showed high similarity to *Streptomyces griseus* (99·5, 99·6 and 99·2 %, respectively) and should be classified as *Streptomyces griseus*. *Streptomyces albidoflavus* exhibited very high similarity (99·9 %) to *Streptomyces sampsonii* and was not differentiated by 16S rRNA gene sequence analysis as shown by Hain *et al.* (1997).

16S rRNA gene sequences are universally known as powerful tools to infer inter- and intrageneric relationships, but are too short to be useful in inferring the phylogenetic relationships between closely related organisms as described above.

Analysis of 16S–23S ITS region sequences

16S–23S ITS regions were used to evaluate whether they can be used in place of 16S rRNA genes to elucidate relationships among closely related *Streptomyces* species. All the strains produced one PCR product containing 16S–23S ITS fragments as determined by agarose electrophoresis. One to eight ITS alleles were selected from each organism. The ITS region sequences were determined for 111 clones of isolates and representative scab-causing strains of *Streptomyces* spp. (Table 1). No tRNA gene was found in the ITS sequences of *Streptomyces* spp. The lengths of ITS sequences (236–287 nt between positions 1531 and 1804 of the *Streptomyces scabiei* ITS region sequence; GenBank no. AB026199) were variable within strains as well as intra- and interspecies, and similarity among sequences was very different (35–100 %). The length and similarity of sequences of ITS alleles are listed in Table 1.

In the phylogenetic analysis based on these sequences, *Streptomyces scabiei* and *Streptomyces europaeiscabiei* clustered within a group. The similarities between type strains of *Streptomyces scabiei* (DSM 41658^T) and *Streptomyces europaeiscabiei* (CFBP 4497^T) varied from 73·0 to 78·5 %. *Streptomyces europaeiscabiei* was differentiated from *Streptomyces scabiei* by three gaps (positions 1600, 1631 and 1662) and by a unique sequence (CTTG→GTAA, position

1658–1661). *Streptomyces scabiei* DSM 41659, 40961 and 40995 were closely related to *Streptomyces europaeiscabiei*, but not to other *Streptomyces scabiei* strains. It is therefore considered that *Streptomyces scabiei* DSM 41659, 40961 and 40995 should be reclassified as *Streptomyces europaeiscabiei*. Three clones of *Streptomyces griseofuscus* were scattered into various groups. The clones of *Streptomyces eurythermus* were also scattered into two groups. *Streptomyces acidiscabies* formed a phyletic line in a group, but *Streptomyces griseofuscus* and *Streptomyces tendae* were also included in this group. *Streptomyces bottropensis* and *Streptomyces stelliscabiei* showed that interspecies sequence homology (94·4–97·1 %) was higher than intrastrain sequence homology (78·8–100 %). Unlike in 16S rDNA analysis, *Streptomyces bottropensis* and *Streptomyces stelliscabiei* were not differentiated from each other. *Streptomyces turgidiscabies* and *Streptomyces reticuliscabiei* were not differentiated as shown by 16S rDNA data. *Streptomyces diastatochromogenes* divided into two phyletic lines and showed more similarity to other species (90·7 % homology with *Streptomyces scabiei* DSM 40962) than intrastrain similarity (84 %). It was also shown that *Streptomyces ipomoeae* is included in the *Streptomyces neyagawaensis* clade, and *Streptomyces sampsonii* and *Streptomyces albidoflavus* were mixed in a phyletic line as shown by 16S rDNA analysis. *Streptomyces scabiei* DSM 40611, 41005 and 41114 were closely related to *Streptomyces griseus* and *Streptomyces setonii*. The phylogenetic tree based on 16S–23S ITS region sequences is available as supplementary data in IJSEM Online.

Except for the differentiation of particular species, such as between type strains of *Streptomyces scabiei* and *Streptomyces europaeiscabiei*, the *Streptomyces* spp. used in this study formed a different phylogenetic lineage between various strains within species (intraspecies). The similarity between strains of species was lower than that between some closely related species. Although the 16S–23S ITS regions have been used as a tool for phylogenetic analysis of bacteria (Gonçalves & Rosato, 2002; Conrads *et al.*, 2002) and a novel database for 16S–23S ITS regions has been created (García-Martínez *et al.*, 2001), this study of intraspecific 16S–23S ITS region sequences of *Streptomyces* spp. has revealed that they are not useful in phylogenetic analysis. In other words, the 16S–23S ITS regions were of no use as a genetic marker to elucidate relationships among the *Streptomyces* spp. used in this study. In other studies (Gürtler & Stanisich, 1996; Hain *et al.*, 1997; Gonçalves & Rosato, 2002; Conrads *et al.*, 2002) and in this study, it is suggested that 16S–23S ITS region sequences are a powerful tool for phylogenetic analysis of Gram-negative bacteria, but not of Gram-positive bacteria, especially *Streptomyces* spp.

In conclusion, based on phylogenetic analysis of 16S rRNA gene sequences, most of the *Streptomyces* spp. isolated from potato scab lesions in this study were classified into *Streptomyces scabiei* and *Streptomyces acidiscabies*. It is thought that Korean isolate KJO61, *Streptomyces reticuliscabiei* and Finnish strains related to *Streptomyces turgidiscabies* should be analysed by other genetic methods or DNA–DNA relatedness to clarify their taxonomic position. From the data obtained in the 16S–23S ITS region sequence analysis, it was revealed that *Streptomyces europaeiscabiei* could be clearly differentiated from *Streptomyces scabiei*. However, it was confirmed that ITS regions are not useful in the phylogenetic analysis of *Streptomyces* spp.

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