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Identification and characterization of *Streptomyces alkaliscabies* sp. nov.

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Abstract

A new bacterial species is described, for which we propose the name *Streptomyces alkaliscabies.* This organism causes a scab disease of potatoes (*Solanum tuberosum*). The alkaline scab symptoms caused by this organism are indistinguishable from the symptoms of common scab caused by *Streptomyces scabies.* In culture, *S. alkaliscabies* is distinct from other scab-causing *Streptomyces* species, having flexuous spore chains and grey mass colour with non-diffusible pigments. *S. alkaliscabies* grows on agar media at pH 7-11 (versus pH 5.0 for S. *scabies*) and has high utilization of all tested sugars including mannitol, sucrose, glucose, raffinose, fructose, melibiose, cellulose, maltose and lactose as carbon source. It is intolerant to 1% phenol and salinity (NaCl at 5, 6 and 7%), melanoid pigments are produced from peptone-iron agar and tyrosine, it degrades arbutin and allantoin and is sensitive to thallium acetate at 100 µg/ml, streptomycin (25 µg/ml), oleandomycin (100µg/ml) and penicillin (10 IU/ml) and tolerates thallium acetate at 10 µg/ml. The 16S-23S ITS region sequence was 466 bp in length and showed relatively low sequence similarity, less than 60%, with other strains. A phylogenetic tree constructed on the basis of 16S rRNA gene sequence showed that the *Streptomyces* spp. that cause potato scab, including *S. scabies, S. acidiscabies, S. turgidiscabies, S. reticuliscabie, S. stelliscabiei, S. chiniscabies* and *S. europaeiscabies*, constitute unique branches. It is evident that strain 8 (*S. alkaliscabies*) forms a distinct phyletic line from known pathogenic strains.

Key words: *Streptomyces scabies*, *Streptomyces alkaliscabies*, potato scab, taxonomy.

Introduction

Potato scab disease has long been recognized as one of the most recalcitrant diseases afflicting potatoes worldwide [1-6]. Scab disease harms a broad range of root crops, including potato, sweet potato, radish, carrot, sugar beet and burdock, with potato scab disease especially causing large economic losses [7]. Diseased potato tubers exhibit characteristic dark-brown, corky lesions. The ugly symptoms of the disease reduce the market value of crops, causing economic difficulties for potato producers [8].

The causative agents of potato scab disease are multiple species of the genus *Streptomyces*. *Streptomyces scabies*, *Streptomyces acidiscabies* and *Streptomyces turgidiscabies* are the most studied and well-known causal agents [9-12]. To date, these are the only three species of potato scab pathogens reported in Japan. Recent studies have shown a correlation between the amounts of these pathogens in soils and the incidence of the disease [13, 14], suggesting that decreasing the quantity of the pathogens in soils could mitigate scab disease damage.

Different *Streptomyces* spp. (more than 14) are all able to cause scab diseases on potato all over the word [1] and differ from each other and from *S. scabies* in their morphological and physiological cultural practices either in laboratory or in the field [15]. The identification and taxonomy of *Streptomyces* spp. has been based on morphological, physiological and pathological tests *in vitro* and *in vivo* [4]. Selectivity to types and quantity of antibiotics and to various concentrations of antibiotics was very important for isolation of *S. scabies* from lesions [16]. Three phenotypic groups have been divided by Faucher *et al.* [17]. Isolation of the first group characterized by tea to brown colonies, grey spores, borne on spiral chains, production of melanin,utilization of L-arabinose, D-fructose, D-mannitol, rhamnose, sucrose, D-xylose and raffinose as sole carbon source and sensitivity to streptomycin. These isolates were referred to *S. scabies*.

Materials and Methods

Isolation of Streptomyces spp.: Potato tubers with scab lesions were obtained from potato field in western desert in Egypt, cultivated in calcareous soils (Fig. 1). The method of Loria and Davis [18] was used to isolate actinomycetes associated with these lesions. Spores from colonies were picked off and streaked onto starch-nitrate media [19]. To ensure pure cultures, developing

Figure 1. Typical scab lesions on a field grown potato in western desert in Egypt.

colonies were purified at least three times by serial passage on the same medium [19]. All isolates were maintained on the same medium. The following additional strains were included as reference strains: *Streptomyces scabies* ATCC 23282 [9], *S. acidiscabies* ATCC 49004 [10], *S. turgidiscabies* ATCC 700248 [11], *S. europaeiscabies* CFBP 4497[1], *S. griseus* ATCC 10246 [20], *S. tendae* ATCC 19812 [21] and *S. setonii* ATCC 25497 [22].

Morphology and pigment production: Spore chain morphology was assessed as described by Shirling and Gottlieb [23]. Spore ornamentation was examined by scanning electron microscope (SEM). The sample used for scanning electron microscopy was prepared by the method of Eguchi *et al*. [24]. Transmission electronic microscope (TEM) was also used. The presence of melanoid pigments on tyrosine and peptone-yeast extract-iron agar media[23] was recorded. Colour determinations were made for the mass colour of mature, sporulating aerial mycelium, substrate mycelium, and diffusible pigment using medium 1, 2, 3, 4, 5 described by International Streptomyces Project (ISP). The pH sensitivity of both the substrate mycelium and diffusible pigments was assessed by the addition of acid or alkali [23, 25].

Determination of degradation activity: The degradation of allantoin was recorded using the media and methods of Gordon and Horan [20] and Gordon *et al*. [26]. Arbutin degradation was determined on a medium containing (%, w/v) yeast extract, 0.3; ferric ammonium citrate, 0.05 and agar, 0.75 [27].

Determination of carbon source utilization: The ability to use sole carbon sources was examined on carbon utilization agar [23]. Carbon sources were added at 1.0% (w/v) to basal agar medium. The basal medium contained (g l^{-1}): $(NH_4)_2SO_4$, 2.64 g; KH_2PO_4, 2.28 g; $K_2H_2PO_4.3H_2O$, 5.65g; $MgSO_4.7H_2O$, 1 g; $CuSO_4.5H_2O$, 4.6 g; $FeSO_4.7H_2O$, 1.1 g; $MnCl_2.H_2O$, 7.9 g; $ZnSO_4.7H_2O$, 1.5 g; agar, 15 g [28].

Determination of antibiotic activity: The toxicities of antibiotics and other inhibitory compounds were determined on modified Bennett agar medium composed of (g/l) beef extract, 4 g; yeast extract, 1 g; trypton, 2 g; glycerol, 10; agar, 15. The toxic compounds tested were selected from those used by Williams *et al*. [27]. Compounds were added to melted agar after autoclaving. Readings were taken at 1, 2, 3 and 7 days, and presence of growth was scored as positive.

Determination of temperature and pH sensitivity: Tolerance to temperature was tested on modified Bennett agar. The minimum pH that allowed growth was determined on media described by Williams *et al*. [27].

Determination of pathogenicity by tuber slice assay: Potato tubers free from surface disease were washed and sterilized for 1 min with 10% bleach (0.06 % NaClO). A tissue disk (2.0 cm in diameter and 1.0 cm in height) was bored from the tuber and placed on moist filter paper in a Petri plate. Pathogenicity of isolate (S8) was examined and compared with *S. scabies*, and photographed as described by Hao *et al*. [15]. *Streptomyces* sp. inoculum was prepared from the culture grown on oatmeal agar medium for 7 days. *Streptomyces* spore suspension (10 µl) or 3 mm diameter agar plug from *Streptomyces* agar culture was placed at the center of the potato tuber disks. The disks were incubated in a moist closed container for 5 to 7 days at 22-24°C in the dark. Necrosis of the tuber slices was evaluated as the area of necrosis minus the area of inoculation.

DNA extraction and purification: All strains were grown in YEME medium [29] at 28°C for 48 h with shaking. The cultures were harvested by centrifugation at 3000 *g* for 10 min, and the mycelia pellets were rinsed and suspended in 10 ml cold TS (50 mM Tris, 15% sucrose, w/v; pH 8.0). The tubes containing the suspensions were placed in boiling water for 3 min and in liquid nitrogen for 3 min. This operation was repeated three times to weaken the cell walls. Lysozyme (5 mg ml^{-1}) and EDTA (0.1 M) were added and the mixture incubated at 37°C for 1 h. Proteinase K (0.2 mg/ml) was added and incubation continued for 15 min. SDS was added to a concentration of 2% (w/v) and the homogenate was incubated at 37°C overnight, until full lysis was achieved. DNA was purified as described by Brenner *et al.* [30].

Analysis of the 16S-23S ITS region: The 16S-23S ITS region sequences of the test isolates were derived from fragments generated by PCR with the following primer sets: AM44 (5´-CTT CGG GGT GTG GGG ACT CAC-3´, corresponding to positions 1113-1134 of the 16S rDNA in *Streptomyces lividans'* TK21) and AM42 (5´-CAA GGG CAT CCA CCG T-3´, corresponding to positions 21-35 (reverse complement) of the 23S rDNA in L1 strains [31,32]). Amplification was carried out in 20 µl of reaction system containing 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 0.1% Triton X-100, 1.0 to 2.0 mM $MgCl_2$, 250 µM each dNTP, 10 pmol each primer, *Taq* DNA polymerase at 5 U/µl, 2 to 25 ng DNA template, and MilliQ water to bring the volume to 20 µl. The thermal cycle was set to the following conditions: initial denaturation at 95°C for 3 min; followed by 40 cycles of denaturing at 95°C for 20 s, annealing at 60°C for 30 s, and extension at 72°C for 2 min; and ending with a 4°C hold. The PCR product was verified by agarose gel electrophoresis and purified by using the QIAquick PCR product purification kit (Qiagen Science, MD). The PCR product was sequenced. The 16S rDNA ITS region sequences were aligned. A phylogenetic tree (an unrooted tree and all branch lengths) was constructed using ClustalX version 2 [33, 34]. To generate the tree, sequences of 16S rDNAs of all the collected isolates were aligned.

Results

Isolation of Streptomyces spp. from scabbed potato: In this study, eight different strains were isolated in pure cultures from scab lesions. The eight strains were cultivated on five standard agar media (ISP media 1-5) (Table 1). Strains 1 and 2 were similar in the phenotypic characters with buff aerial mycelium, yellow to yellowish brown substrate mycelium, spiny spore surface with no diffusible pigments. Strains 3 and 7 were also similar in possessing grey aerial mycelium, spiny spore surface and produce purple diffusible pigment on ISP medium 1only. Strains 3 and 7 differ in substrate mycelium colour, which are yellowish brown in strain 3, while grey on ISP medium 1, buff on media ISP media 2-4 and yellow on ISP medium 5 for strain 7. Strain 4 characterized by rose aerial mycelium on all ISP media except ISP medium 4 which is buff, with spiny spore surface and no diffusible pigments. Strain 4 has yellowish brown substrate mycelium on all ISP media tested.

Table 1. Phenotypic characterization of the eight *Streptomyces* strains grown on different selective media.

Character	Strain	Starch nitrate Agar	Inorganic salt-starch	Oat meal Agar	Yeast-malt extract Agar	Glycerol-Asparagin Agar
Aerial mycelium	S1	Buff	Buff	Buff	Buff	Buff
	S2	Buff	Buff	Buff	Buff	Buff
	S3	Grey	Grey	Grey	Grey	Grey
	S4	Rose	Buff	Rose	Rose	Rose
	S5	Grey	White/Grey	White/Grey	White/Grey	White/Grey
	S6	Grey	White/Grey	White/Grey	White/Grey	White/Grey
	S7	Grey	Grey	Grey	Grey	Grey
	S8	White/Grey	White/Grey	Grey	Grey/Green	Grey
Substrate mycelium	S1	Yellow	Yellow/Brown	Yellow/Brown	Yellow/Brown	Yellow/Brown
	S2	Yellow	Yellow/Brown	Yellow/Brown	Yellow/Brown	Yellow/Brown
	S3	White/Yellow	Yellow/Brown	Yellow/Brown	Yellow/Brown	Yellow/Brown
	S4	Yellow/Brown	Yellow/Brown	Yellow/Brown	Yellow/Brown	Yellow/Brown
	S5	Grey	Grey	Grey	Grey	Yellow
	S6	Grey	Grey	Grey	Grey	Yellow
	S7	Grey	Buff	Buff	Buff	Yellow
	S8	Grey	Grey/Green	Grey	Grey/Green	Grey
Spore Surface	S1	Spiny	Spiny	Spiny	Spiny	Spiny
	S2	Spiny	Spiny	Spiny	Spiny	Spiny
	S3	Spiny	Spiny	Spiny	Spiny	Spiny
	S4	Spiny	Spiny	Spiny	Spiny	Spiny
	S5	Smooth	Smooth	Smooth	Smooth	Smooth
	S6	Smooth	Smooth	Smooth	Smooth	Smooth
	S7	Spiny	Spiny	Spiny	Spiny	Spiny
	S8	Smooth	Smooth	Smooth	Smooth	Smooth
Diffusible pigments	S1	None	None	None	None	None
	S2	None	None	None	None	None
	S3	Purple	None	None	None	None
	S4	None	None	None	None	None
	S5	None	None	None	None	None
	S6	Purple	None	None	None	None
	S7	None	None	None	None	None
	S8	None	None	None	None	None

Strains 5 and 6 were phenotypically similar to each other and to standard *S. scabies* in having smooth spore surface, grey aerial and substrate mycelium and no diffusible pigments (Table 1).

Description of Streptomyces alkaliscabies sp. nov.: The morphological properties of strain (8) (*Streptomyces alkaliscabies*) was consistent with its assignment to the genus *Streptomyces* [27]. After 14 days of growth, *S. alkaliscabies* forms highly branched aerial mycelium, which was well developed. Smooth, cylindrical spores with 1.9 -2.1 mm long were born in long, flexuous spore chains (Fig. 2). Growth of aerial and substrate mycelium was found on all ISP media tested. Spore colour developed from white to grey after 10 days (Fig. 2). This strain is fully differentiated from *S. scabies* and from other phytopathogenic *Streptomyces* species causing common scab (*S. acidoscabies, S. turgidiscabies, S. caviscabies, S. europaeiscabies, S. stelliscabies, S. reticuliscabies*). *S. alkaliscabies* share almost all the morphological and biochemical characteristics that are important in the identification of *Streptomyces* species. It does not grow in the presence of different NaCl concentrations, phenol, penicillin, oleandomycin and streptomycin, but uses manitol, sucrose, glucose, raffinose, fructose, melibiose, cellulose, maltose and lactose. It degrades arbutin and allantoin. It does not grow at 45°C and pH 4.5 (Table 2). Sporangial and sclerotial formation structures were not observed, while chlamydospores are present. Necrosis was observed 36 h after inoculation on potato tuber disks inoculated with either strain 8 (*S. alkaliscabies*) or *S. scabies.* Necrotic lesions continued to expand over a period of 7 days. No necroses or any colour changes of the tuber disks were observed when non-inoculated agar was used (Fig. 3). The pathogenicity test confirmed that *S. alkaliscabies* produces common scab lesions.

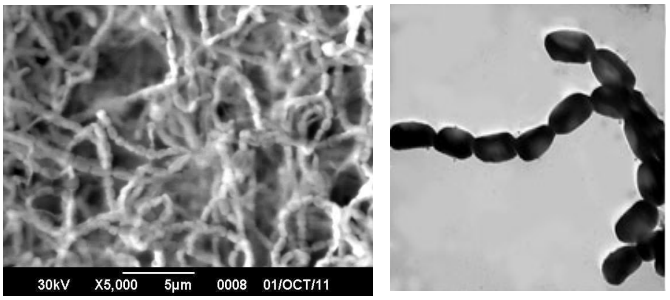

Figure 2. Morphological properties of *Streptomyces alkaliscabies* strain. Chain morphology by SEM (left) and spore surface ornamentation by TEM (right).

The pH tolerance of the strain 8 revealed that the minimum pH for growth was 5.0, associated with weak necrotic symptoms on the potato tuber disks. The growth and pathogenicity of the strain begins to increase gradually with an increase in the alkalinity up to pH 11. The experiment reveals exclusively, that the strain is alkali-resistant or alkalophilic strain. This character is another distinctive feature in addition to the previously described phenotypic, physiologic and pathogenic characters (Table 3).

Consensus primers AM44 and AM42 successfully amplified

Table 2. Phenotypic and physiological characterization that differentiate *S. alkaliscabies* (S8) from other *Streptomyces* species.

Characteristics	*S. al*	*S. s*[a]	*S. ac*	*S. tu*	*S. e*	*S. st*	*S. g*	*S.te*	*S. se*
Spore mass colour[b]	G	G	W	G	G	G	G	G	G
Chain morphology[c]	F	S	RF	RF	S	S	RF	RF	RF
Spore surface[d]	Sm	Sm	Sm	Sm	ND	ND	Sm	Sp	Sm
Diffusable pigments[e]	None	None	R/Y	None	ND	ND	None	Y/O	None
Utilization[f]									
Mannitol	+	+	+	ND	+	+	+	+	+
Sucrose	+	+	+	ND	+	+	+	+	+
Glucose	+	+	+	ND	+	+	+	+	+
Raffinose	+	+	-	+	+	+	-	-	-
Fructose	+	+	+	ND	+	+	+	+	+
Melibiose	+	ND	ND	ND	ND	ND	-	ND	-
Cellulose	+	ND	ND	ND	ND	ND	ND	ND	ND
Maltose	+	ND	ND	ND	ND	ND	ND	ND	ND
Lactose	+	ND	ND	ND	ND	ND	ND	ND	ND
Growth in 1% phenol	-	-	+	-	ND	ND	+	+	+
Melanin from peptone agar	+	+	ND	ND	+	+	ND	ND	ND
Melanin from tyrosine agar	+	+	-	ND	+	+	-	-	-
Degradation									
Arbutin	+	+	-	ND	ND	ND	+	-	+
Allantoin	+	-	-	ND	ND	ND	ND	ND	-
Growth in NaCl 5, 6, 7%	-	+	-	-	ND	ND	+	+	+
Growth at 45°C	-	ND	ND	ND	ND	ND	+	+	-
Growth at pH 4.5	-	-	+	+	ND	ND	-	+	-
Sensitivity test (growth)									
Thallium acetate (100 µg/ml)	-	-	-	-	ND	ND	+	-	+
Thallium acetate (10 µg/ml)	+	+	-	-	ND	ND	+	+	+
Penicillin (10 IU/ml)	-	-	+	ND	+	+	+	+	+
Oleandomycin (100 µg/ml)	-	-	-	-	ND	-	+	+	+
Streptomycin (25 µg/ml)	-	-	+	-	-	-	+	+	+

+, positive reaction; -, negative reaction. [a] Data of *S. scabies*, *S. acidoscabies*, *S. turgidiscabies*, *S. caviscabies*, *S. europaeiscabiei*, *S. stelliscabiei*, *S. reticuliscabi*, *S. griseus*, *S.tendae*, *S. setonii* [1, 9-11] Type strain of S.s, *S. scabies*; S.ac, *S. acidoscabies*; S.tu, *S. turgidiscabies*; S.e, *S. europaeiscabiei*; S.st, *S. stelliscabiei*; S.g, *S. griseus*; S.te, *S.tendae*; S.se, *S. setonii*. [b] G, grey, W, white. [c] F, flexuous; RF, Recti-flexuous; S, Spiral. [d] Sm, smooth; Sp, Spiny. [e] R/Y, red/yellow; Y/O, yellow/orange. [f] ND, not determined.

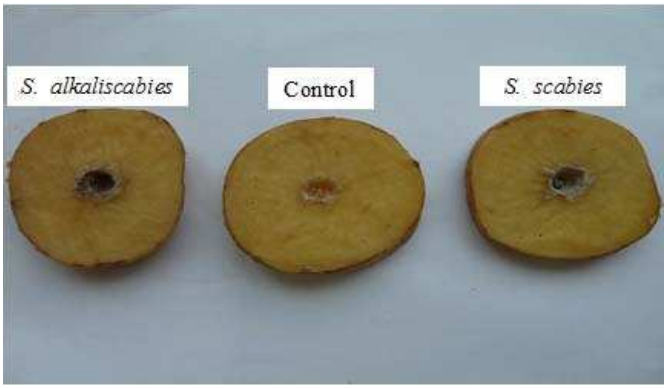

Figure 3. Pathogenicity assay on tuber slices: necrotic halos produced around the agar plugs taken from 5-day-old oatmeal agar cultures of *Streptomyces alkaliscabies* (left), agar only (middle), and *Streptomyces scabies* (right).

Table 3. Effect of pH on growth and pathogenicity of *Streptomyces alkaliscabies.*

PH	*S. alkaliscabies* (S8)	
	Growth	Pathogenecity
3	-	-
4	-	-
5	+	+
6	++	+
7	+++	+
8	+++	+
9	+++	+
10	+++	+
11	+++	+
12	-	-

+, weak growth; ++, moderate growth; +++, heavy growth.

the 16S-23S ITS region. The 16S-23S ITS region sequence was 466 bp in length and considerable sequence variation was observed between species (Fig. 4). Strain 8 (*S. alkaliscabies*) showed relatively low sequence similarity, less than 60%, with other strains (Table 4).

A phylogenetic tree constructed on the basis of 16S rRNA gene sequence showed that the *Streptomyces* spp. that cause potato scab, including, *S. scabies*, *S. acidiscabies*, *S. turgidiscabies*, *S. reticuliscabie*, *S. stelliscabiei*, *S. chiniscabies* and *S. europaeiscabiei*, constitute unique branches. It is evident that strain 8 (*S. alkaliscabies*) forms a distinct phyletic line from known pathogenic strains (Fig. 5).

Discussion

Plant pathogenic *Streptomyces* species has been classified by phenotypic characteristics such as colour of spores and substrate mycelium, spore chain morphology, pigment production, sugar utilization of International *Streptomyces* Project (ISP), and resistance to inhibitory compounds [9, 10, 23]. In recent years, phylogenetic analyses using rRNA operon and DNA relatedness, and guanine and cytosine composition (GC) have been extensively used for identification at species level [1, 11, 35, 36]. In this study eight *Streptomyces* strains grown on five selective standard media (ISP media 1-5) showed differences in their morphological characteristics. Strains 5 and 6 were identical to *S. scabies* in possessing grey aerial mycelium, smooth spore surface and no diffusible pigments. Non scabies but scab producing strains are usually less virulent and have been identified as *S. griseus* [20, 21, 37], *Streptomyces olivacean* [21], *S. aureofaciens* [38] and *S. flaveolus* [22].

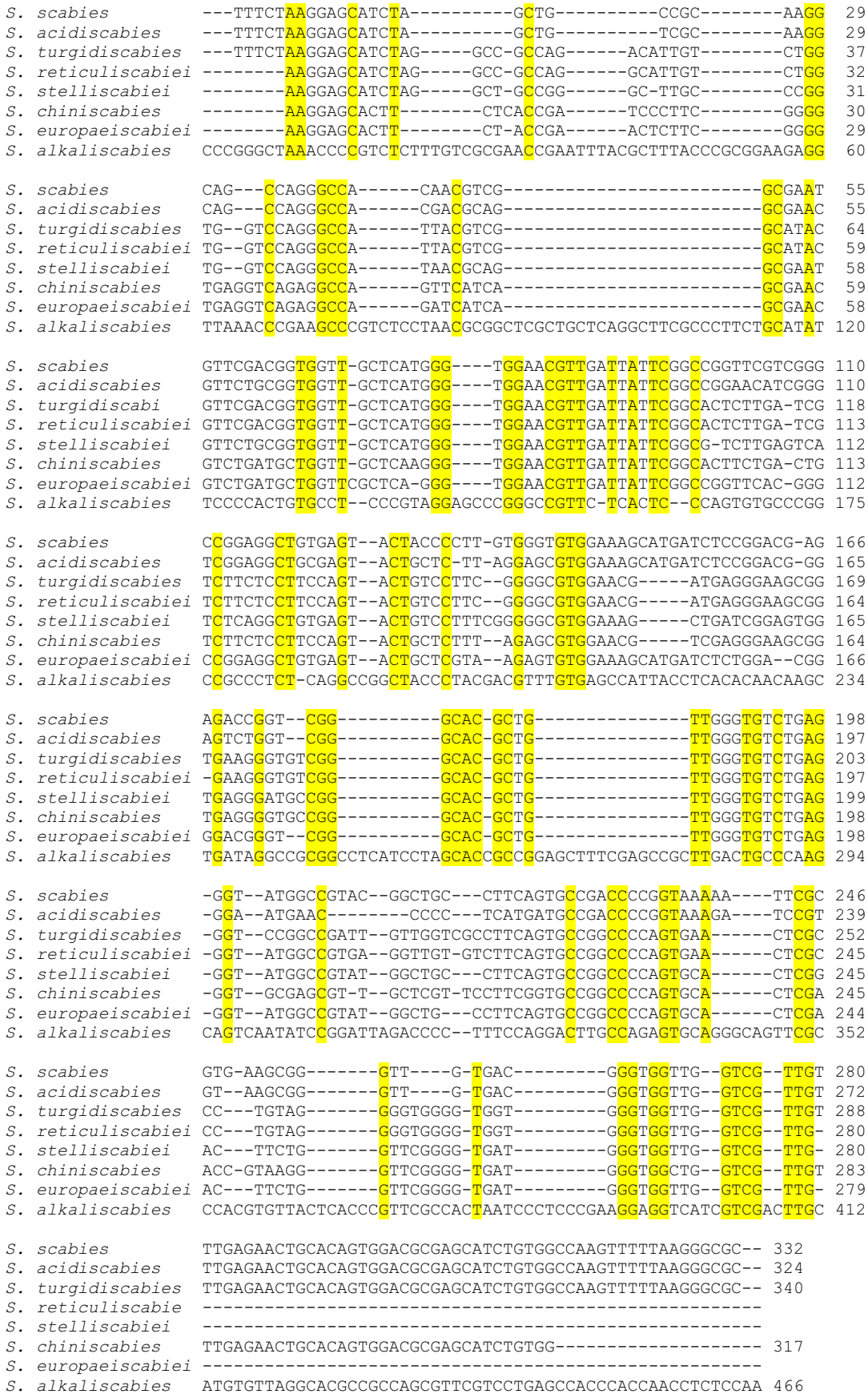

Figure 4. Multiple sequence alignment of nucleotide sequences of 16S rRNA gene sequences. The 16S rDNA sequences of the Streptomyces isolates were downloaded from the GenBank with accession numbers and additional resource information: *Streptomyces scabies* (AB042775), *Streptomyces acidiscabies* (AB041130), *Streptomyces turgidiscabies* (AB041147), *Streptomyces reticuliscabies* (AY296981), *Streptomyces chiniscabies* (AY845137), *Streptomyces stelliscabies* (AY296993), *Streptomyces europaeiscabies* (AY296985). Alignment was done with ClustalW 2.0 software [33]

Table 4. Relative similarity of sequence of 16S-23S ITS region of *S. alkaliscabies* sp. nov. with other potato pathogenic *Streptomyces* species sequences obtained from gene data bank.

Species	*S. scabies*	*S. acidiscabies*	*S. turgidiscabies*	*S. reticuliscabie*	*S. stelliscabiei*	*S. chiniscabies*	*S. europaeiscabiei*
S. acidiscabies	79						
S. turgidiscabies	90	79					
S. reticuliscabie	75	96	73				
S. stelliscabiei	71	83	71	81			
S.chiniscabies	80	82	75	83	77		
S. europaeiscabiei	81	76	75	76	83	83	
S. alkaliscabies	53	57	55	60	53	54	59

The *S. alkaliscabies* (8) strain was proved to be alkaline tolerant to very high pH reaching 11either in growth and pathogenicity via producing necrotic lesions in potato disks. This character is very distinct into the present strain which suggested as a new *S. alkaliscabies*. It was found to be distinct from the previously characterized *S. acidiscabies* ATCC 49004 [10], *S. griseus* ATCC10246 [20], *S. tendae* ATCC 19812 [21], *S. setonii* ATCC 25497 [22] and *S. scabies* ATCC 33282 [9] in being heavily grown on raffinose, producing melanoid pigment from asparagine and tyrosine, degrading allantoin, being intolerant to salinity and 1% phenol, and sensitive to penicillin and streptomycin.

Four differences were distinctive between *S. alkaliscabies* and *S. scabies* ATCC 33282, the latter possesses spiral spore chain, unable to produce melanin from tyrosine, unable to degrade allantoin and intolerant to saline. These distinct differences suggest that these two species are not closely related and their ability to infect plant by scab symptoms developed independently which is in accordance with Lambert and Lorea [9, 10], in case of *S. acidiscabies* (ATCC 49004) and *S. scabies* (ATCC 33282).

The 16S-23S ITS region sequence was 466 bp in length and considerable sequence variations was observed between species. Strain 8 (*S. alkaliscabies*) showed relatively low sequence similarity with other strains, having less than 60%. A phylogenetic tree constructed on the basis of 16S rRNA gene sequence showed that the *Streptomyces* spp. that cause potato scab, including, *S. scabies*, *S. acidiscabies, S. turgidiscabies*, *S. reticuliscabies*, *S. stelliscabies*, *S. chiniscabies* and *S. europaeiscabiei*, constitute unique branches. It is evident that strain 8 (*S. alkaliscabies*) forms a distinct phyletic line from known pathogenic strains. Studies have shown some diversity within strains morphologically identified as *S. scabies*, as well as other phytopathogenic streptomyces, and provided evidence that potato scab is caused by a polyphyletic group of *Streptomyces* spp. [39].

Conclusions

Streptomyces alkaliscabies sp. nov., *alkaliscabies* referring to the ability of the organisms to grow and cause scab of potatoes under alkaline conditions (up to pH 11). The spores are smooth and born in mature flexuous chain containing 5-10 or more spores with 1.9-2.1 µ spore length. No diffusible pigment and melanin pigments produced from asparagine and tyrosine. Fructose, D-glucose, D-mannitol, sucrose, raffinose, cellulose, melibiose, maltose, and lactose are used as carbon sources. It differ in at least 6 characters from the reference standard *Streptomyces* species and in four characters from *S. scabies* ATCC 33282

References

[1]Bouchek-Mechiche, K., Pasco, C., Andrivon, D. and Jouan, B. 2000. Differences in host range, pathogenicity to potato cultivars and response to soil temperature among *Streptomyces* species causing common and netted scab in France. Plant Pathol. **49**:3-10.

[2]Park, D. H., Kim, J. S., Cho, J. M., Hur, J. H. and Lim, C. K. 2003. Characterization of Streptomycetes causing potato scab in Korea. Plant Dis. **87**:1290-1296.

[3]Tanaka, F. 2004. Identification and quantification of pathogens in Japan. In Naito, S., Kondo, N., Akino, S., Ogoshi, A. and Tanaka, F. (eds). Proceedings of the International Potato Scab Symposium. Hokkaido University, Sapporo, pp. 56–65.

[4]Wanner, L. A. 2004. Field isolates of *Streptomyces* differ in pathogenicity and virulence on radish. Plant Dis. **88**:785-795.

[5]St-Onge, R., Goyer, C., Coffin, R. and Filion, M. 2008. Genetic diversity of *Streptomyces* spp. causing common scab of potato in eastern Canada. Syst. Appl. Microbiol. **31**:474-484.

[6]Zhao, W. Q., Liu, D. Q. and Yu, X. M. 2008. First report of potato scab caused by *Streptomyces turgidiscabies* in China. Plant Dis. **92**:1587-1587.

[7]Loria, R., Bukhalid, R. A., Fry, B. A. and King, R. R. 1997. Plant pathogenicity in the genus Streptomyces. Plant Dis. **77**:836-846.

[8]Johnson, D. A. and Powelson, M. L. 2008. Potato Health Management. American Phytopathological Society Press, St. Paul, MN.

[9]Lambert, D. H. and Loria, R. 1989a. *Streptomyces scabies* sp. nov., nom. rev. Int. J. Syst. Bacteriol. **39**:387-392.

[10]Lambert, D. H. and Loria, R. 1989b. *Streptomyces acidiscabies* sp. nov. Int. J. Syst. Bacteriol. **39:**393-396.

[11]Miyajima, K., Tanaka, F., Takeuchi, T. and Kuninaga, S. 1998. *Streptomyces turgidiscabies* sp. nov. Int. J. Syst. Bacteriol. **48**:495-502.

[12]Kers, J., Cameron, K., Joshi, M., Bukhalid, R., Morello, J., Wach, M., Gibson, D. and Loria, R. 2005. A large, mobile pathogenicity island confers plant pathogenicity on *Streptomyces* species. Mol. Microbiol. **55**:1025-1033.

[13]Koyama, O., Manome, A., Kurata, S., Yokomaku, T. and Tanaka, H. 2006. Correlation between *nec1* gene copy number detected in soils by quantitative competitive quenching probe PCR and incidence of potato common scab disease. Microb. Environ. **21**:185-188.

[14]Manome, A., Kageyama, A., Kurata, S., Yokomaku, T., Koyama, O., Kanagawa, T., Tamaki, H., Tagawa, M. and Kamagata, Y. 2008. Quantification of potato common scab pathogens in soil by quantitative competitive PCR with fluorescent quenching based probes. Plant Pathol. **57**:887-896.

[15]Hao, J. J., Meng, Q. X., Yin, J. F. and Kirk, W. W. 2009. Characterization of a new *Streptomyces* strain DS3024 that causes potato common scab. Plant Dis. **93**:1329-1334.

[16]Conn, K. L., Lee, E., Kritzman, G. and Lazarovits, G. 1998. A quantitative method for determining soil populations of *Streptomyces* and differentiating potential potato scab inducing strains. Plant Dis. **82**:631-638.

[17]Faucher, E., Otrysko, B., Paradis, É., Hodge, N. C., Stall, R. E. and Beaulieu, C. 1993. Characterization of streptomycetes causing russet scab in Quebec. Plant Dis. **77**:1217-1220.

[18]Loria, R. and Davis, J. R. 1988. III. Gram-positive bacteria, B. *Streptomyces scabies.* In Schaad, N.W. (ed.). Laboratory Guide for Identification of Plant Pathogenic Bacteria. American Phytopathological Society, St Paul, MN, pp. 114-119.
[19]Matumoto, K. 1979. Sporulating media and preservation of *Streptomyces scabies.* Plant Prot. **33**:461-463.
[20]Gordon, R. E. and Horan, C. 1968. A piecemeal description of *Streptomyces griseus* (Krainsky) Waksman and Henrici. J. Gen. Microbiol. **50**:223-233.
[21]Hütter, R. 1967. Systematik der Streptomyceten unter besonderer Berücksichtigung der von ihnen gebildeten Antibiotica. S. Karger, Basel, Schweiz, New York, 382 p.
[22]Millard, W. A. and Burr, S. 1926. A study of twenty-four strains of *Actinomyces* and their relation to types of common scab of potato. Ann. Appl. Biol. **13**:580-644.
[23]Shirling, E. B. and Gottlieb, D. 1966. Methods for characterization of *Streptomyces* species. Int. J. Syst. Bacteriol. **16**:313-340.
[24]Eguchi, T., Takada, N., Nakamura, S., Tanaka, T., Makino, T. and Oshima, Y. 1993. *Streptomyces-Bungoensis* sp. Nov. Int. J. Syst. Bacteriol. **43**:794-798.
[25]Williams, S. T., Davies, F. L., Mayfield, C. I. and Khan, M. R. 1971. Studies on the ecology of actinomycetes in soil. 11. The pH requirements of streptomycetes from two acid soils. Soil Biol. Biochem. **3**:187-195.
[26]Gordon, R. E., Barnett, D. A., Handerhan, J. E. and Pang, C. H. N. 1974. *Nocardia coeliaca*, *Nocardia autotrophica* and *Nocardia* strain. Int. J. Syst. Evolut. Microbol. **24**:54-63.
[27]Williams, S. T., Goodfellow, M., Alderson, G., Wellington, E. M. H., Sneath, P. H. A. and Sackin, M. J. 1983. Numerical classification of *Streptomyces* and related genera. J. Gen. Microbiol. **129**:1743-1813.
[28]Pridham, T. G. and Gottlieb, D. 1948. The utilization of carbon compounds by some Actinomycetales as an aid for species determination. J. Bacteriol. **56**:107-114.
[29]Chater, K. F., Hopwood, D. A., Kieser, T. and Thompson, C. J. 1982. Gene cloning in *Streptomyces*. Curr. Top. Microbiol. Immunol. **96**:69-95.
[30]Brenner, D. J., McWorter, A. C., Leete Knutson, J. K. and Steigerwalt, A. G. 1982. *Escherichia vulneris*: a new species of Enterobacteriaceae associated with human wounds. J. Clin. Microbiol. **15**:1133-1140.
[31]Jensen, M. A., Webster, J. A. and Straus, N. 1993. Rapid identification of bacteria on the basis of polymerase chain reaction amplified ribosomal DNA spacer polymorphisms. Appl. Environ. Microbiol. **59**:945–952.
[32]Mehling, A., Wehmeier, U. F. and Piepersberg, W. 1995. Nucleotide sequences of streptomycete 16S ribosomal DNA: Towards a specific identification system for streptomycetes using PCR. Microbiology **141**:2139-2147.
[33]Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., McGettigan, P. A., McWilliam, H., Valentin, F., Wallace, I. M., Wilm, A., Lopez, R., Thompson, J. D., Gibson, T. J. and Higgins, D. G. 2007. ClustalW and ClustalX version 2.0. Bioinformatics **23**:2947-2948.
[34]Saitou, N. and Nei, M. 1987. The neighborjoining method - a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. **4**:406-425.
[35]Kreuze, J. F., Suomalainen, S., Paulin, L. and Valkonen, J. P. T. 1999. 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. Phytopathology **89**:462-469.
[36]Takeuchi, T., Sawada, H., Tanaka, F. and Matsuda, I. 1996. Phylogenetic analysis of *Streptomyces* spp. causing potato scab based on 16S rRNA sequences. Int. J. Syst. Bacteriol. **46**:476-479.
[37]Corbaz, R. 1964. Etude des streptomycetes provoquant la gale commune de la pomme de terre. Phytopathol. Z. **51**:351-360.
[38]Archuleta, J. G. and Easton, G. D. 1981. The cause of deep-pitted scab of potatoes. Am. Potato J. **58**:385-392.
[39]Healy, F. G. and Lambert, D. H. 1991. Relationships among *Streptomyces* spp. causing potato scab. Int. J. Syst. Bacteriol. **41**:479-482.

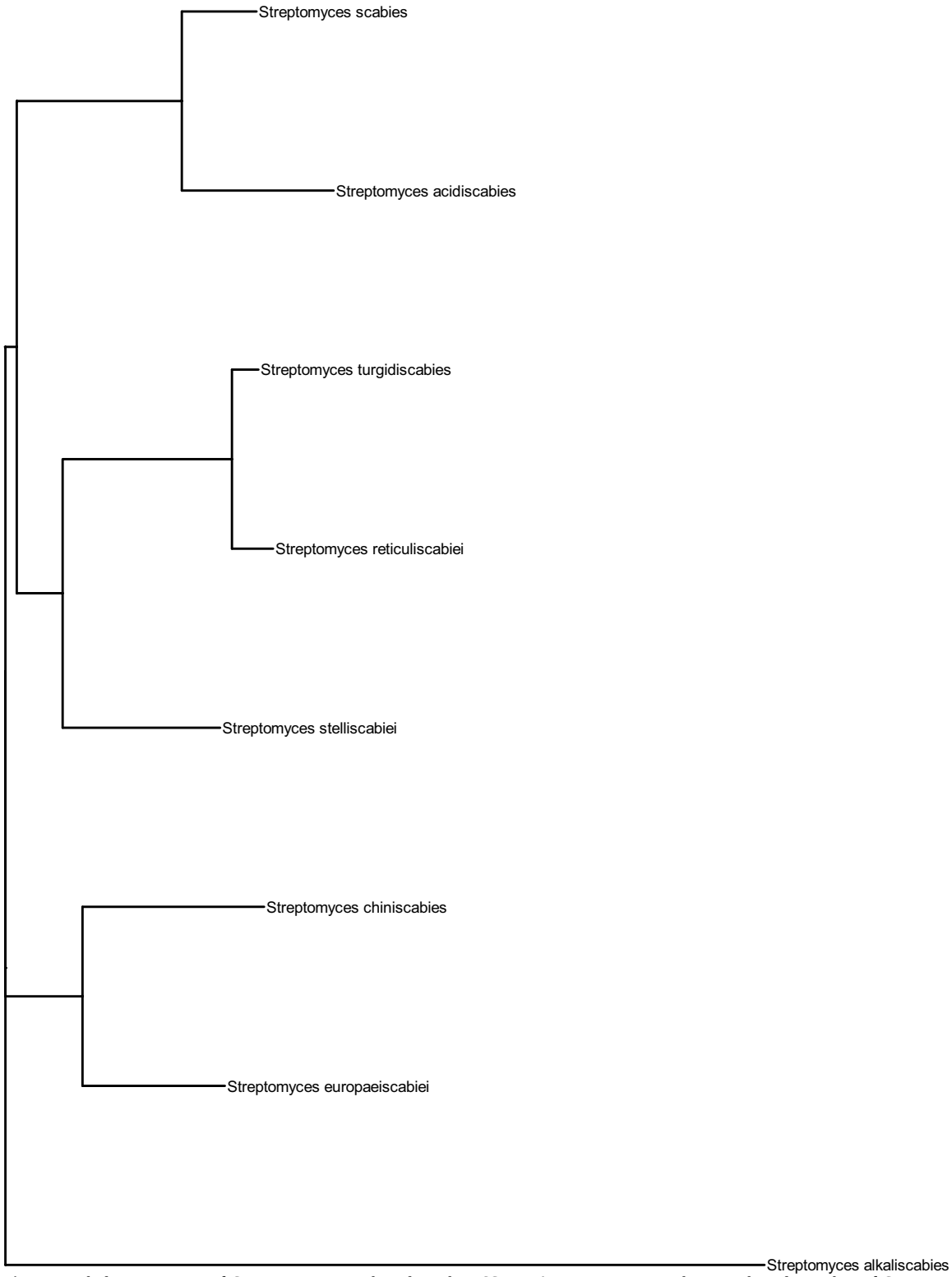

Figure 5. Phylogenetic tree of Streptomyces spp. based on the 16S rRNA gene sequences showing the relationship of Streptomyces alkaliscabies to other Streptomyces spp. The 16S rDNA sequences of the Streptomyces isolates were downloaded from the GenBank with accession numbers and additional resource information: *Streptomyces scabies* (AB042775, ATCC 33282), *Streptomyces acidiscabies* (AB041130), *Streptomyces turgidiscabies* (AB041147), *Streptomyces reticuliscabiei* (AY296981), *Streptomyces chiniscabies* (AY845137), *Streptomyces stelliscabiei* (AY296993), *Streptomyces europaeiscabiei* (AY296985).