



Major *Streptomyces* species associated with fissure scab of potato in South Africa including description of *Streptomyces solaniscabiei* sp. nov

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Abstract *Streptomyces* species are the causal agents of several scab diseases on potato tubers. A new type of scab symptom, caused by *Streptomyces* species, was observed in South Africa from 2010 onwards. The disease was initially thought to be caused by a single *Streptomyces* species, however, subsequent isolations from similar symptoms on other potato tubers revealed diversity of the *Streptomyces* isolates. The objective of this study was to characterise these isolates in order to determine what are the major species involved in the disease. This was done by sequencing and

phylogenetic analyses of the 16S rDNA as well as five housekeeping genes, investigation of growth on different culture media, standard phenotypic tests and scanning electron microscopy of culture morphology. The presence of the pathogenicity island (PAI) present in plant pathogenic *Streptomyces* species was also investigated. The genomes of eight isolates, selected from the three main clades identified, were sequenced and annotated to further clarify species boundaries. Three isolates of each of the three main clades were also inoculated onto susceptible potato cultivars in order to establish the pathogenicity of the species. The results of the phylogenetic and genome analyses revealed that there are three main species involved, namely, *Streptomyces werraensis*, *Streptomyces pseudogriseolus* and a novel *Streptomyces* species that is described here as *Streptomyces solaniscabiei* sp. nov., with strain FS70^T (= PPPPB BD 2226^T = LMG 32103^T) as the type strain. The glasshouse trial results showed that all three of the *Streptomyces* species are capable of producing fissure scab symptoms. None of the *Streptomyces* isolates from fissure scab contained the full PAI and the mechanism of disease initiation still needs to be determined. Genomic comparisons also indicated that *S. gancidicus* Suzuki 1957 (Approved Lists 1980) is a later heterotypic synonym of *S. pseudogriseolus* Okami and Umezawa 1955 (Approved Lists 1980).

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Introduction

Streptomyces species are causal agents of some of the most widespread and economically important diseases in potato production worldwide (Braun et al. 2017; Wanner 2006). Common scab is caused by species in the *Streptomyces scabiei* complex. Other related *Streptomyces* species are the causal agents of netted scab in Europe (Scholte and Labruyère 1985) and russet scab in North America and Japan (Faucher et al. 1993; Oniki et al. 1986). All of these diseases are caused by complexes of *Streptomyces* species and have large variations in symptom expression (Bouchek-Mechiche et al. 2000; Wanner 2006).

Plant pathogenicity in the genus *Streptomyces* has been demonstrated to be based on a specific toxin, thaxtomin, which is produced by pathogenic strains causing common scab. Genes encoding enzymes for the synthesis of thaxtomin (*txtA*, *txtB*, *txtC*, and *nos*) are located in a pathogenicity island (PAI) (Loria et al. 2006). Other genes encoding virulence factors also found on the PAI in pathogenic *Streptomyces* species include tomatinase (*tomA*) and the necrosis-inducing protein (*nec1*) (Wanner 2006). Thaxtomin inhibits cellulose biosynthesis and is able to induce plant cell hypertrophy in growing plant tissues (Fry and Loria 2002; Scheible et al. 2003), thereby causing the scabs on potato tubers (Fiers et al. 2012). Tomatinase is predicted to be involved in plant defence suppression (Kers et al. 2005) by detoxification of tomatin, an antimicrobial saponin (Roldán-Arjona et al. 1999). The *nec1* gene encodes a small necrogenic protein with an unknown plant cell target (Bukhalid et al. 1998) that may be involved in plant defence suppression (Joshi et al. 2007).

The availability of whole genome sequences for thousands of organisms have enabled researchers to study fields such as the evolution of organisms (Chandra and Chater 2014) and the occurrence of horizontal gene transfer (HGT) more effectively (Armijos-Jaramillo et al. 2017). HGT has been shown to be responsible for the emergence of several new plant and human pathogens (De la Cruz and Davies 2000; Friesen et al. 2006; Garcia-Vallvé et al. 2000).

In *Streptomyces*, it has been demonstrated that PAI present in pathogenic *Streptomyces* species could confer pathogenicity to non-pathogenic organisms during mating experiments in the laboratory (Kers et al. 2005; Zhang et al. 2016; Zhang and Loria 2017). Bukhalid et al. (2002) also presented evidence of HGT between *Streptomyces* species causing the emergence of multiple plant-pathogenic species. This mechanism can therefore lead to non-pathogens acquiring pathogenicity genes and causing diseases that previously did not exist.

The genus *Streptomyces* has undergone extensive revisions and currently contains around 700 validly named species (<http://www.bacterio.net/streptomyces.html>), of which most are saprophytic and present in large numbers in the soil (Janssen 2006; Pierzynski et al. 2005). There have been numerous name changes and taxonomic revisions of the species in this genus since the first description of the common scab pathogen as *Oöspora scabies* by Thaxter (1892). One of the most comprehensive reviews was initiated by the International *Streptomyces* Project (ISP) committee, which was established in 1963. This provided a standard set of tests to use in describing *Streptomyces* species (Shirling and Gottlieb 1966, 1968). However, since the advent of DNA sequencing (examples applied to *Streptomyces* include: Labeda et al. 2012; Labeda et al. 2017) and more recently whole genome sequencing (Sutcliffe et al. 2012; Jain et al. 2018; Parks et al. 2018), these methods have been superseded in the description of bacterial species. However, many *Streptomyces* descriptions still include results of biochemical tests, as these may be an indication of useful secondary metabolites produced by the *Streptomyces* species.

In South Africa, Gouws and McLeod (2012) described a new disease of potatoes, associated with *Streptomyces* species. The symptoms on potato tubers consisted of deep longitudinal cracks with scab-like lesions (Fig. 1a) and was termed “fissure scab”. These blemishes differ from common scab (Fig. 1b) and growth cracks (Fig. 1c) in the star shaped fissures that are commonly associated with fissure scab. The causal agent was identified as a *Streptomyces* species related to *Streptomyces vinaceus*, *S. malachiticus*, *S. wer-raensis*, *S. cyaneus* and *S. pseudogriseolus*. The four isolates that were sequenced during the initial description of the disease were identical (Gouws and McLeod 2012), leading researchers to believe that a single

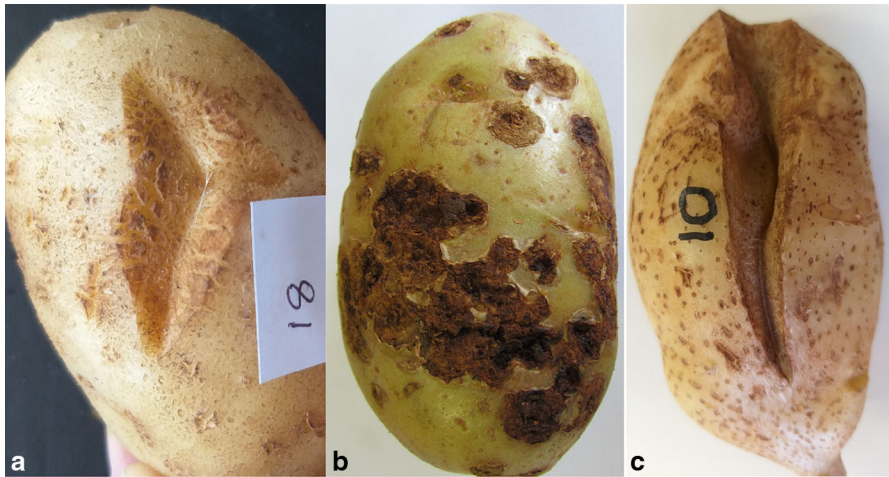


Fig. 1 Potato tubers with a. fissure scab, b. common scab and c. growth crack

Streptomyces species was responsible for the disease. However, subsequent isolations from fissure scab symptoms over the last eight years have revealed considerable morphological variation in the *Streptomyces* isolates obtained from these symptoms. Further investigations into the causal agents of fissure scab was therefore initiated and the results of these studies are presented here.

Materials and methods

Sample collection and isolation

Potato tuber samples, displaying scab symptoms as described by Gouws and McLeod (2012), were collected from various potato production regions in South Africa. Isolation of *Streptomyces* from potatoes was done according to Loria and Davis (1989). The potatoes were washed and surface disinfected with 70% ethanol for 1 min. The potatoes were then rinsed thoroughly with sterile distilled water. The surface of the lesions was removed and discarded. The tissue under the scab lesion was cut into smaller pieces and crushed in 1 mL of sterile distilled water. Approximately 0.1 mL of the crushed tissue suspension was streaked onto Inorganic Salt Starch agar (ISSA—ISP medium 4 according to Shirling and Gottlieb (1966)). Plates were incubated at 28 °C for 5 days. Colonies resembling *Streptomyces* species were selected and purified by streaking on ISSA plates. Isolates were preserved as spore suspensions in 20% glycerol at -

80 °C in the *Streptomyces* bacterial collection at the Agricultural Research Council-Vegetables, Industrial and Medicinal Plants (ARC-VIMP).

DNA extraction, PCR amplification and sequencing

All *Streptomyces* isolates obtained from symptomatic tubers were grown on ISSA for 5 days at 30 °C, after which DNA was extracted for PCR and sequencing using the Zymo Research fungal/bacterial DNA isolation kit (Zymo Research Corporation, Irvine CA, USA). DNA was amplified by PCR using commonly used primers for bacterial characterisation (Bukhalid et al. 2002; Guo et al. 2008). The 16S rDNA was amplified and sequenced for all isolates and housekeeping genes were amplified and sequenced for isolates belonging to the major groups identified based on 16 S rDNA analyses.

The PCR reactions consisted of 1 µL of template DNA, 1 µL of each primer, 12.5 µL of GC Tempase Mastermix II (Ampliqon A/S, Denmark) made up to 25 µL with PCR grade water. Conditions for the PCR included an initial denaturing at 95 °C for 15 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing of primers at 55 °C (for 16S rDNA), 63 °C (for *rpoB* and *recA*), 65 °C (for *trpB*), 60 °C (for *atpD*), and 58 °C (for *gyrB*) for 20 s, elongation at 72 °C for 30 s and a final extension step at 72 °C for 10 min. PCR products were visualized on 1% agarose gels, stained with ethidium bromide.

Forward and reverse strands of PCR products were sequenced by Inqaba Biotec (Pretoria, South Africa). The forward and reverse sequences were assembled and consensus sequences generated using CLC Genomics Workbench 10.0 (<https://www.qiagenbioinformatics.com/>). Sequences of type strains of *Streptomyces* species were downloaded from GenBank (<https://blast.ncbi.nlm.nih.gov/>) and aligned with newly generated sequences with the online version of MAFFT v.7 (<http://mafft.cbrc.jp/alignment/server/index.html>).

Phylogenetic analyses

Phylogenetic analyses were conducted using PhyML 3.0 (Guindon and Gascuel 2003). Initial Maximum Likelihood (ML) trees of individual genes were constructed to check whether the different gene regions resulted in congruent tree topologies. After checking for congruence, the protein coding genes were combined into a single dataset. Selection of models of nucleotide substitution for the PhyML analyses, implementing the Akaike information criterion (AIC), was determined with jModeltest 2.1.7 (Guindon and Gascuel 2003; Darriba et al. 2012). Phylogenetic trees were mid-point rooted and bootstrap analysis was performed to determine branching point confidence intervals (1000 replicates). An initial analysis of 16S rDNA with all described *Streptomyces* species and all isolates obtained from fissure scab was conducted in order to determine which species are closely related to the fissure scab isolates. All subsequent analyses only included the selected subset of isolates.

Pathogenicity trial

Initial screening of the three major groups of *Streptomyces* isolates, identified based on 16S rDNA sequencing, were conducted to select virulent isolates for use in a greenhouse trial. Potato tubers cv. Mondial, were purchased from a supermarket in Pretoria. The tubers were washed thoroughly with water, disinfected in 1% sodium hypochlorite (NaClO) for 5 min, rinsed with sterile distilled water and air dried under a laminar flow hood. Inoculum was prepared for each isolate by making spore suspensions in sterile water in 20 mL McCartney bottles from mycelia and spores grown on ISSA plates for 5 days.

Disinfected tubers were sliced into 7 mm thick slices with a sterilized knife and placed on top of moistened sterile filter paper in 90 mm plastic Petri dishes. A 10 μ L aliquot of inoculum was pipetted in the centre of each tuber slice. Sterile water was included as a negative control. Petri dishes were placed in boxes lined with moist paper towels and incubated at 25 °C in the dark for 5 days. Treatments were replicated three times and arranged in a completely randomized design. The tubers were evaluated visually for the presence and size of the necrotic area on the tuber slice.

The pathogenicity of *Streptomyces* strains was investigated using two cultivars known to be susceptible to fissure scab, namely, Mondial and Innovator. Pots (25 cm diameter) were sterilized with 1% sodium hypochlorite and rinsed with sterile water. Compost was sterilized using a soil pasteurizer at 300 °C for 30 min and sterilized pots were filled halfway with the compost. One potato tuber was placed on top of the compost in each pot, after which the pots were filled with silica sand. The pots were maintained in a greenhouse at 25–28 °C and irrigated three times a week. Three isolates from each of the three main *Streptomyces* clades identified in the phylogenetic analysis were selected based on results from the tuber slice assay. The isolates were grown on ISSA for 7 days and scraped off to make a spore suspension to be used as inoculum.

At the tuber initiation stage, the pots were inoculated with the spore suspensions and placed in a randomized complete block design with 10 replicates. The control pots were inoculated with sterile distilled water. Potatoes were harvested three months after planting and evaluated for fissure scab symptoms using a custom rating scale:

Lesion length (LT):

- 1 = 1–25% of tuber length.
- 2 = 25–50% of tuber length.
- 3 = 50–75% of tuber length.
- 4 = 75–100% of tuber length.

Lesion depth (LD):

- 1 = lesion depth is superficial.
- 2 = lesion depth is medium deep.
- 3 = lesion depth is very deep.

Scab Index = (LTxLD)/100.

Streptomyces species were re-isolated from potatoes with fissure scab symptoms. The isolates were purified and identified by DNA extraction, PCR and

sequencing, as described above, in order to fulfil Koch's postulates. Data were analysed with SAS software (SAS Institute, Inc., 1999) to determine significant differences between treatments.

Genome sequencing and annotation

Isolates were selected for genome sequencing based on the results of the 16S rDNA and tuber slice assays. Two isolates from each of the three main clades were chosen to sequence initially and an additional two isolates from clade three were sequenced based on the results from the first genome sequencing, resulting in eight *Streptomyces* isolates genomes being sequenced. The sequencing was performed on an Illumina HiSeq 2500 producing 2*125 bp paired-end reads. Quality control was done using FastQC v0.11.5 (Andrews 2010) and Trimmomatic v0.36 (Bolger et al. 2014). Genome assemblies were generated using SPAdes v3.12.0 (Bankevich et al. 2012) and the quality of the resulting assemblies evaluated by QUAST v4.6.3 (Gurevich et al. 2013). The assemblies were annotated with RAST (Overbeek et al. 2013). Average nucleotide identity (ANI) and average amino acid identity (AAI) matrices were calculated using the enveomics toolbox (Rodriguez-R and Konstantinidis 2016).

To elucidate possible mechanisms of pathogenicity, local BLAST searches for commonly found pathogenicity associated genes in *Streptomyces* were conducted on CLC Genomics Workbench. Annotated genomes were searched for virulence and pathogenicity associated genes.

Morphology and phenotypic analysis

Isolates from the three main clades identified by sequencing were selected for morphological investigation. Colony morphology was investigated on Yeast extract-Malt extract agar (YMA, ISP medium 2), Oatmeal agar (OA, ISP medium 3), ISSA, Glycerol-Asparagine agar (GA, ISP medium 5) and Tyrosine agar (TA, ISP medium 7). Isolates were streaked onto the different culture media, incubated at 30 °C in the dark and investigated for colony colour and pigment production after 7 and 14 days.

Spore chain morphology of isolates grown on OA for 7 days at 30 °C was investigated by scanning electron microscopy (SEM). Material was prepared by immersion in 2.5% glutaraldehyde in 0.075 M

phosphate buffer (pH 7) for 1 h. The specimens were washed three times (10 min each) in 0.075 M phosphate buffer and fixed in 0.5% aqueous osmium tetroxide for 1–2 h. The material was washed three times in distilled water and dehydrated (10 min each) in 30%, 50%, 70%, 90%, and three times in 100% ethanol. Hexamethyldisilazane was used to dry the material, after which it was mounted on aluminium stubs and coated with carbon. A Zeiss Crossbeam 540 FEG SEM was used to visualize the material.

Carbohydrate utilisation was determined using a 1% concentration of each carbon source (L-arabinose, D-fructose, D-glucose, I-inositol, D-mannitol, raffinose, rhamnose, sucrose and D-xylose) added to the carbon utilization medium (ISP medium 9—Shirling and Gottlieb 1966) and rated for growth 14 days after incubation at 25 °C. The optimum temperatures for growth were assessed using ISP medium 1 at a range of 5–45 °C, with 5 °C intervals. Tolerance to sodium chloride was established using basal medium 5339 (10 g casein peptone L⁻¹, 5 g yeast extract L⁻¹, 15 g agar L⁻¹) supplemented with 0–15% (w/v) sodium chloride with 2.5% intervals. The pH tolerance of isolates was tested on YMA plates at pH levels from 4 to 12. The pH levels of 4, 5.5, 7, 8.5 and 10.0 were adjusted with NaOH or HCl before autoclaving, and pH 11.5 was adjusted from pH 10 after autoclaving.

Results

Sample collection and isolation

A total of 142 *Streptomyces* isolates were obtained from tubers with symptoms that resemble fissure scab. The symptomatic tubers were collected from all of the major potato production regions in South Africa.

Phylogenetic analyses

Newly generated DNA sequences from this study are available from GenBank with accession numbers MK934844-MK934992 and MK956209-MK956788. Isolates included in the phylogenetic analyses are listed in Table 1. Alignment of sequences for individual datasets yielded 1,367 bp (16S), 496 bp (*atpD*), 428 bp (*gyrB*), 504 bp (*recA*), 540 bp (*rpoB*) and 571 bp (*trpB*). The combined dataset of the 5 housekeeping genes yielded a dataset of 2,539 bp. The

Table 1 GenBank accession numbers of *Streptomyces* isolates included for analyses in this study

Species	Strain*	GenBank accession numbers						Genome
		16S	<i>atpD</i>	<i>gyrB</i>	<i>recA</i>	<i>rpoB</i>	<i>trpB</i>	
<i>S. werraensis</i>	FS39	MK934920	MN410458	MK956276	MK956493	MN410504	MN410521	
	FS73	MK934946	MK956758	MK956289	MK956521	MK956396	MK956632	
	FS96	MK934970	MK956780		MK956544	MK956416	MK956654	
	FS97, P PPPB BD 2230	MK934971	MK956781		MK956545	MK956417	MK956655	SAMN13430414
	O57, P PPPB BD 2229	MK934988	MK956785	MK956309	MK956559	MK956431	MK956669	SAMN13430417
<i>S. pseudogriseolus</i>	FS33	MK934914	MK956734	MK956271	MK956488	MN410499	MN410516	
	FS38	MK934919	MN410457	MK956275	MK956492	MN410503	MN410520	
	FS41	MK934922	MN410459	MK956277	MK956495	MN410505	MN410522	
	FS94, P PPPB BD 2232	MK934968	MK956778		MK956542	MK956414	MK956652	SAMN13430413
	N92, P PPPB BD 2231	MK934979	MK956784	MK956308	MK956552	MK956424	MK956662	SAMN13430416
<i>S. solaniscabiei</i>	FS66, P PPPB BD 2225	MK934939	MK956751	MK956282	MK956514	MK956389	MK956625	SAMN16577100
	FS70 ^T , P PPPB BD 2226, LMG 32,103	MK934943	MK956755	MK956286	MK956518	MK956393	MK956629	SAMN13430412
	FS75, P PPPB BD 2227	MK934948	MK956759	MK956291	MK956523	MK956398	MK956634	SAMN16577101
	FS82	MK934955	MK956766	MK956298	MK956530	MK956404	MK956641	
	N26, P PPPB BD 2228	MK934975	MN410463		MK956548	MK956420	MK956658	SAMN13430415

* FS, N, O – Isolates stored in the *Streptomyces* bacterial collection at the ARC-VIMP, Pretoria, South Africa. P PPPB BD – Plant Pathogenic and Plant Protecting Bacteria Culture Collection, National Collection, ARC-PHP, Pretoria, South Africa. LMG – BCCM/LMG Bacteria Collection, University of Gent, Belgium

best substitution model for both 16S and combined datasets was GTR + I + G.

An initial 16S rDNA ML tree consisting of all *Streptomyces* species and all fissure scab isolates was constructed (Fig. S1 supplementary material). The majority of the isolates (62%) obtained from fissure scab symptoms grouped into three clades. Clade 1 (24 isolates, 17%) grouped with *S. werraensis*, clade 2 (26 isolates, 18%) grouped close to *S. pseudogriseolus* and *S. gancidicus*, while clade 3 (39 isolates, 27%) grouped close to *S. flaveolus*. The other 53 isolates grouped into 37 different clades and these smaller groupings were not investigated further in the current study as the focus was on the major groups associated with the observed symptoms.

The three most virulent isolates per clade as identified by tuber slice assay, as well as an additional two isolates per clade, were included in the

phylogenetic analyses presented. The exception is clade 1 where FS29 was not included in the phylogenetic analyses as the *gyrB* gene could not be amplified. The tree topologies for the individual housekeeping genes were all congruent (data not presented) and showed concordance with the 16S rDNA tree topology (Fig. 2). The combined tree (Fig. 3) confirmed that the clade 1 isolates belong to species *S. werraensis* with a bootstrap support of 99%. Clade 2 isolates grouped close to *S. pseudogriseolus*, *S. rubiginosus* and *S. gancidicus*, and clade 3 isolates grouped close to, but clearly distinct from, *S. flaveolus* with a bootstrap support of 99%.

Pathogenicity trial

The three isolates from each of the three main phylogenetic clades causing the largest lesions in the

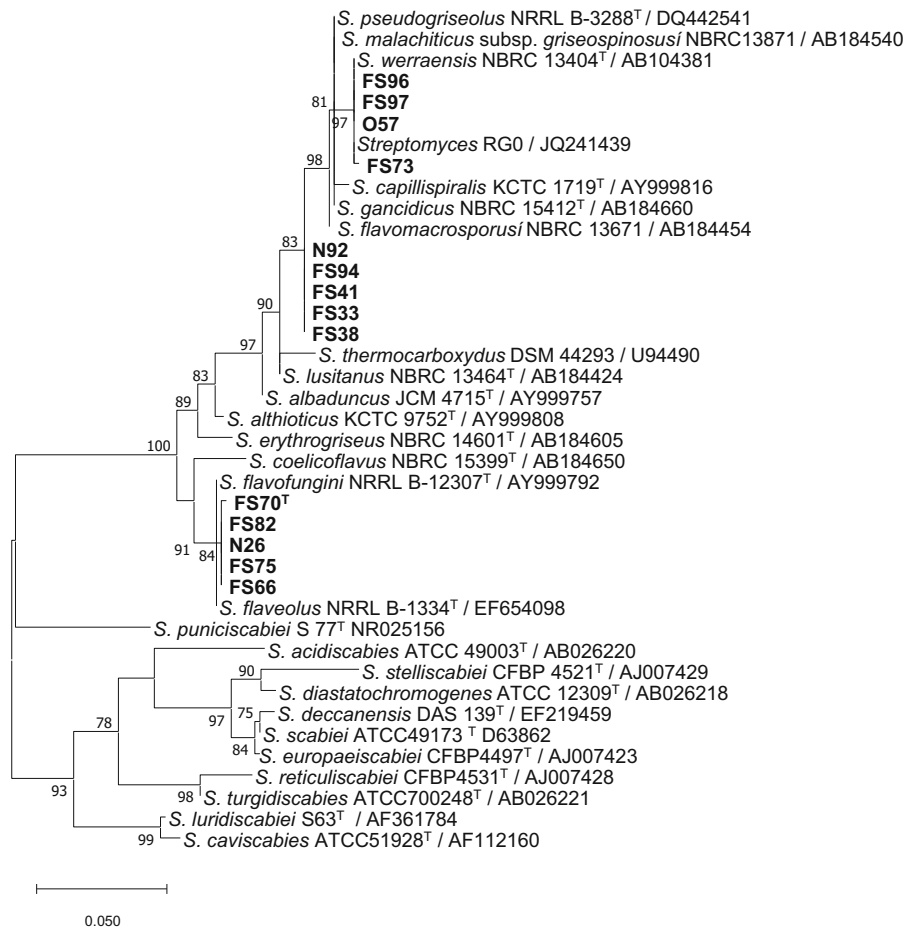


Fig. 2 Midpoint rooted tree of *Streptomyces* species based on partial sequence of the 16S rDNA gene region

tuber slice assay were selected to use to test for pathogenicity in the glasshouse trial. These were isolates O57, FS29, FS97 (clade 1); FS33, FS94, N92 (clade 2); and N26, FS70 and FS75 (clade 3).

Isolates from all three clades were able to cause symptoms similar to those seen in fissure scab (Fig. 4). The results from the glasshouse trial disease ratings are given in Fig. 5. In general, infections on Innovator showed a higher scab index than Mondial, however it is not a statistically significant difference. Isolates FS97 (clade 1) and N26 (clade 3) resulted in a significantly higher scab index on Innovator than on Mondial ($p < 0.05$). However, there was a large variation in the scab index within the same treatments and treatments that differed significantly from the control are indicated with an asterisk in Fig. 5.

Re-isolation and identification of pathogens from diseased tubers confirmed that the inoculated

organisms were responsible for the disease symptoms observed.

Genome sequencing and annotation

Genomes were sequenced at 100 × coverage and assembled into 82, 121, 174, 118, 99, 85, 130, 113 contigs for isolates O57, N92, N26, FS66, FS75, FS97, FS94 and FS70, respectively. The Whole Genome Shotgun project data has been deposited at DDBJ/ENA/GenBank under the accession numbers SAMN16577101, SAMN16577100 and SAMN13430412-SAMN13430417.

The ANI and AAI values in comparison with closely related *Streptomyces* genomes are shown in Fig. 6; however, no genome sequence for *S. werraensis* was available. The values confirmed that clade 3 (isolates N26, FS66, FS70 and FS75) is a distinct

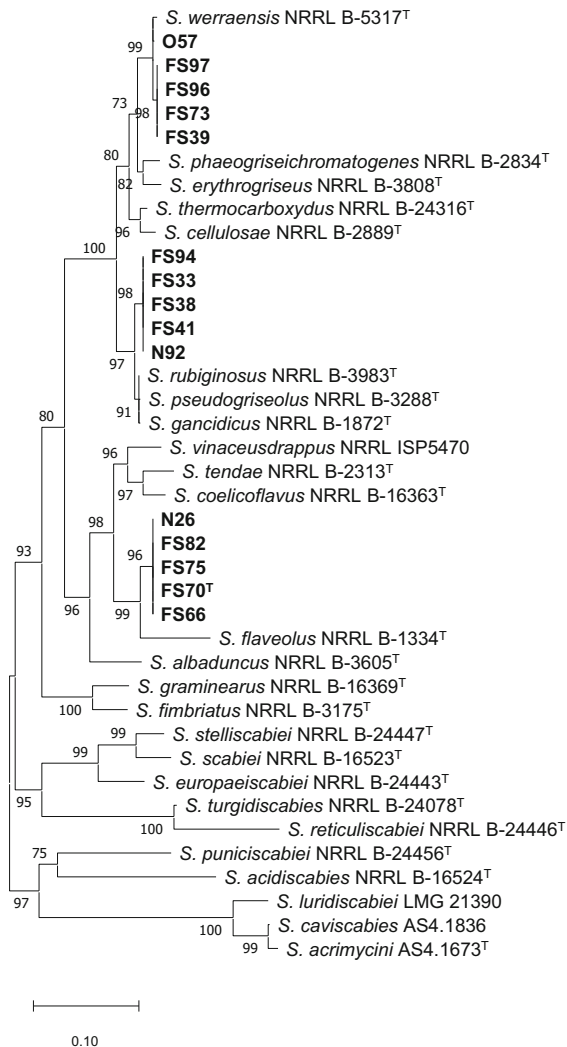


Fig. 3 Midpoint rooted tree of combined house-keeping genes *atpD*, *gyrB*, *recA*, *rpoB*, *trpB* of *Streptomyces* species

species from *S. flaveolus* with 84% ANI and 78% AAI. Clade 2 isolates (isolates N92 and FS94) are similar to *S. pseudogriseolus* and *S. gancidicus* which belong to one species as these isolates share 99% AAI and ANI. Therefore *S. gancidicus* Suzuki 1957 (Approved Lists 1980) is considered a later heterotypic synonym of *S. pseudogriseolus* Okami and Umezawa 1955 (Approved Lists 1980).

The PAI commonly found in plant pathogenic *Streptomyces* species were not present in any of the eight genomes. However, all eight of the isolates contained pathogenicity related genes that were annotated as homologues of the *Staphylococcal* pathogenicity islands (SaPI). These genes include

the Heat shock protein 60 family chaperone GroEL, tmRNA-binding protein SmpB and methionine ABC transporter substrate-binding protein. The isolates of clade 3 also contained the Golgi-associated plant pathogenesis-related protein 1 gene. These annotations were generated by RAST, and so more research is needed to elucidate these gene functions in *Streptomyces* for better annotation of the genomes.

Morphology and phenotypic analysis

Culture morphology on OA is shown in Fig. 2a-c (supplementary material). Isolates belonging to clade 3 produced yellow diffusible pigments on all culture media (Fig. S2c), which made it possible to distinguish these cultures from clades 1 (Fig. S2a) and 2 (Fig. S2b). Some cultures in clade 2 produced a reddish-brown pigment on OMA. SEM revealed that strains from clades 1 (Fig. 7a) and 3 (Fig. 7e) had mycelia in open loops (*Retinaculiaperti*), while those from clade 2 (Fig. 7c) formed simple spirals (*Spirales*). The spore surfaces of strains from all three clades were spiny (Fig. 7b, d, f), with some spores of clade 1 strains exhibiting a warty surface (Fig. 7b). Although the culture morphology of members of clades 1 and 2 were similar, the distinct spirals of strains in clade 2 and the profusion of single spores from members of clade 1 made it possible to distinguish between these clades.

Isolates from clades 1 and 3 were found to be able to utilize L-arabinose, D-fructose, D-glucose, I-inositol, D-mannitol, raffinose, rhamnose, sucrose and D-xylose as sole carbon sources. Isolates from clade 2 were able to utilize L-arabinose, D-glucose, I-inositol, D-mannitol, rhamnose, and D-xylose; however, utilization of sucrose, fructose and raffinose were doubtful (\pm). Isolates from all three clades were found to be able to grow between pH 5.0–pH 11 and to be tolerant of NaCl up to 7.5% but were inhibited by 10% NaCl. Members of clade 3 were found to be able to grow between 10 and 45 °C, with optimal growth between 30 and 35 °C.

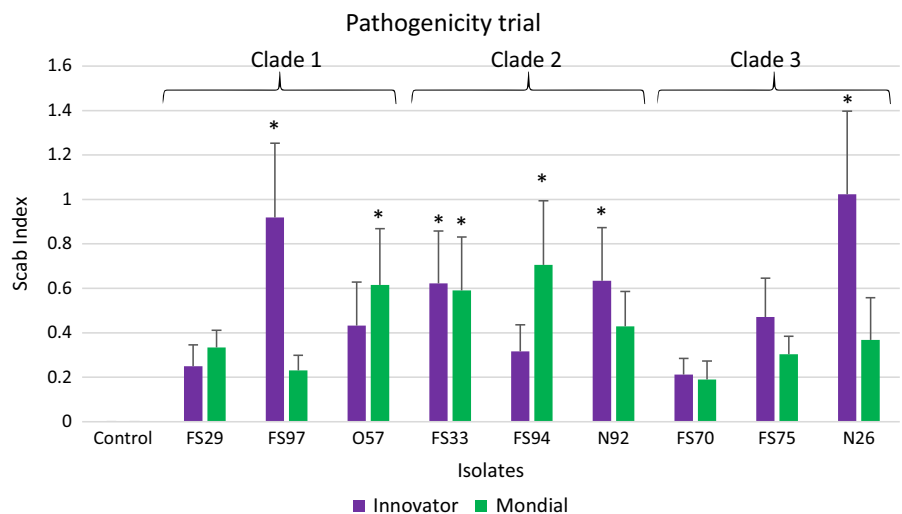
Discussion

This is the first comprehensive survey to identify the *Streptomyces* isolates associated with fissure scab in South Africa. The three main clades identified,



Fig. 4 Symptoms on potato tubers after inoculation with *Streptomyces* species. Top row cv. Innovator and bottom row cv. Mondial

Fig. 5 Results from glasshouse trial showing scab index on cv. Innovator and Mondial. Error bars indicate the standard error and treatments that differed significantly ($p < 0.05$) from the control is indicated with an asterisk (*)



comprising 62% of the isolates obtained from fissure scab symptoms from 2010–2018, were identified as belonging to *S. werraensis* (clade 1), *S. pseudogriseolus* (clade 2) and clade 3, representing a novel species, described below as *Streptomyces solaniscabiei* sp. nov. Full genome sequences were generated for eight isolates selected from the three species. Glasshouse trials with isolates of the three species revealed that all three species are capable of causing fissure scab symptoms on potato cvs. Mondial and Innovator. The PAI genes present in the common scab pathogens, comprised of thaxtomin, tomatinase and

nec1, are absent in most of the isolates, with only the *nec1* gene being present in some isolates of *S. solaniscabiei* (data not shown).

Horizontal gene transfer of the PAI has been proven to occur in *Streptomyces* species and to lead to the emergence of new pathogenic species (Bukhalid et al. 2002; Kers et al. 2005; Zhang et al. 2016; Zhang and Loria 2017). However, this PAI is absent in the fissure scab isolates, and this leads to the question of the mechanism of disease development. The genomes that were generated will be investigated further for indications on which genes may be responsible for

◀ **Fig. 6** **a** Average Nucleotide Identity matrix, **b**. Average Amino acid Identity matrix of *Streptomyces* species, based on full genome sequences

initiating the cracks that are the main characteristic of the fissure scab symptoms.

The advent of DNA sequencing and more recently genome sequencing has removed the necessity of doing scores of phenotypic tests in the hope of finding morphological and phenotypic differences in order to describe a novel bacterial species. These tests are still useful in finding novel biochemical products produced by the ubiquitous *Streptomyces* genus, however, time and money spent by taxonomists will be greatly

reduced without having to do these tests (Sutcliffe et al. 2012), while increasing the rate at which species can be described. As more full genomes become available, the use of the ANI and AAI statistics of these genomes will aid in making decisions on where to delimit species. The 16S rDNA as well as the combined phylogenetic trees in this study were inconclusive on the identities of clades 2 and 3. However, the ANI and AAI matrixes conclusively showed that clade 2 strains are members of *S. pseudogriseolus*, while clade 3 strains are not members of *S. flaveolus*, but represent a novel species for which the name *S. solaniscabiei* sp. nov. is proposed. The phenotypic, phylogenetic and genomic data

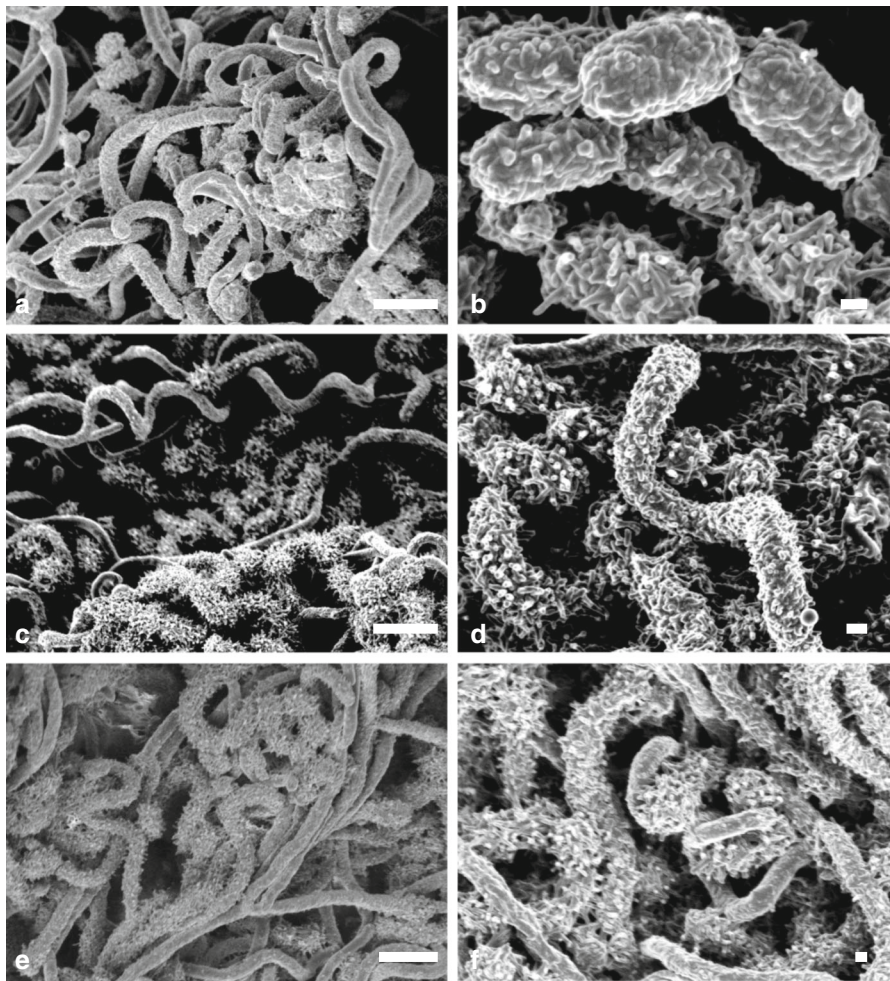


Fig. 7 SEM images of *Streptomyces* isolates obtained from fissure scab symptoms: **a**. *S. werraensis* mycelia, **b**. *S. werraensis* spores, **c**. *S. pseudogriseolus* mycelia in spirals,

d. *S. pseudogriseolus* spores and spirals, **e**. clade 3 (*S. solaniscabiei* sp. nov.) mycelia, and **f**. clade 3 spores. Scale bars: **a**, **c**, **e** = 2 μm, **b**, **d**, **f** = 200 nm

support this proposal and the formal description of this new species follows below.

Description of *Streptomyces solaniscabiei* sp. nov.

Streptomyces solaniscabiei (so.la.ni.sca.bi.e'i. L. neut. n. *solanum*, nightshade, and the genus name of the potato (*Solanum tuberosum*); L. fem. n. *scabies*, scab, mange; N.L. gen. n. *solaniscabiei*, of the potato scab).

Gram-stain positive, aerobic, non-motile, alkali tolerant and thermotolerant. Forms hyphae that are extensively branched with aerial hyphae that differentiate into open loops of spores (*Retinaculiaperti*). Good growth on ISP 3 and ISP 4, poor growth on ISP 2 and 5, and moderate growth on ISP 7 media. Colonies on ISP 3 are white initially, turning light grey after sporulation, with white margins. Diffusible yellow pigment discolours media. Able to utilize L-arabinose, D-fructose, D-glucose, I-inositol, D-mannitol, raffinose, rhamnose, sucrose and D-xylose as sole carbon sources. Grows from pH 5.0–pH11. Grows well in the presence of 0–5% NaCl and can tolerate up to 7.5% NaCl, but were inhibited at concentrations of 10% and higher. Grows between 10 and 45 °C, with optimal growth between 30 °C and 35 °C.

The strains FS70^T, N26, FS75 and FS80 were isolated from potato tubers with fissure scab symptoms in the Limpopo Province of South Africa. The type strain is FS70^T (= PPPPB BD 2226^T = LMG 32103^T). The GenBank accession number for the 16S rDNA gene sequence of FS70^T is MK934943 and the genome accession number is SAMN13430412.

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Authors' contribution Conceptualization: M. Cloete, Methodology: M. Cloete, E. Cruywagen, R. Pierneef, D. Labeda; Formal analysis and investigation: K. Chauke, Z. Nkosi, E. Cruywagen, R. Pierneef; Writing—original draft preparation: E. Cruywagen, K. Chauke; Writing—review and

editing: M. Cloete, D. Labeda, R. Pierneef, Z. Nkosi; Funding acquisition: M. Cloete; Supervision: M. Cloete, E. Cruywagen.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Availability of data and material All sequence data have been deposited on GenBank and the type culture of the novel species was deposited into 2 international culture collections.

References

- Andrews (2010) Babraham bioinformatics-FastQC a quality control tool for high throughput sequence data. URL: bioinformatics. babraham. ac. uk/projects/fastqc/
- Armijos-Jaramillo V, Santander-Gordón D, Soria R, Pazmiño-Betancourth M, Echeverría MC (2017) A whole genome analysis reveals the presence of a plant PR1 sequence in the potato pathogen *Streptomyces scabies* and other *Streptomyces* species. *Mol Phylogenet Evol* 114:346–352
- Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV (2012) SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* 19(5):455–477
- Bolger AM, Lohse M, Usadel B (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinform* 30(15):2114–2120
- Braun S, Gevens A, Charkowski A, Allen C, Jansky S (2017) Potato common scab: A review of the causal pathogens, management practices, varietal resistance screening methods, and host resistance. *American J Potato Res* 94(4):283–296
- Bouchek-Mechiche K, Gardan L, Normand P, Jouan B (2000) DNA relatedness among strains of *Streptomyces* pathogenic to potato in France: description of three new species, *S. europaeiscabiei* sp. nov. and *S. stelliscabiei* sp. nov. associated with common scab, and *S. reticuliscabiei* sp. nov. associated with netted scab. *Int J Syst Evol Microbiol* 50(1):91–99
- Bukhalid RA, Chung SY, Loria R (1998) *necl*, a gene conferring a necrogenic phenotype, is conserved in plant-pathogenic *Streptomyces* spp. and linked to a transposase pseudogene. *Mol Plant Microbe In* 11(10): 960–967
- Bukhalid RA, Takeuchi T, Labeda D, Loria R (2002) Horizontal transfer of the plant virulence gene, *necl*, and flanking sequences among genetically distinct *Streptomyces* strains in the Diastatochromogenes cluster. *Appl Environ Microbiol* 68(2):738–744

- Chandra G, Chater KF (2014) Developmental biology of *Streptomyces* from the perspective of 100 actinobacterial genome sequences. *FEMS Microbiol Rev* 38(3):345–379
- Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nat Methods* 9(8):772
- De la Cruz F, Davies J (2000) Horizontal gene transfer and the origin of species: lessons from bacteria. *Trends Microbiol* 8(3):128–133
- Faucher E, Otrysko B, Paradis E, Hodge NC, Stall RE, Beaulieu C (1993) Characterization of *Streptomyces* causing russet scab in Québec. *Plant Dis* 77(12):1217–1220
- Fiers M, Edel-Hermann V, Chatot C, Le Hingrat Y, Alabouvette C, Steinberg C (2012) Potato soil-borne diseases. *A Rev Agron Sustain Dev* 32(1):93–132
- Friesen TL, Stukenbrock EH, Liu Z, Meinhardt S, Ling H, Faris JD, Rasmussen JB, Solomon PS, McDonald BA, Oliver RP (2006) Emergence of a new disease as a result of inter-specific virulence gene transfer. *Nat Genet* 38(8):953–956
- Fry BA, Loria R (2002) Thaxtomin A: evidence for a plant cell wall target. *Phys Mol Plant Path* 60(1):1–8
- García-Vallvé S, Romeu A, Palau J (2000) Horizontal gene transfer in bacterial and archaeal complete genomes. *Genome Res* 10(11):1719–1725
- Gouws R, McLeod A (2012) Fissure scab, a new symptom associated with potato common scab caused by a *Streptomyces* sp. *South Africa Plant Dis* 96(8):1223
- Guindon S, Gascuel O (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Syst Biol* 52(5):696–704
- Guo Y, Zheng W, Rong X, Huang Y (2008) A multi-locus phylogeny of the *Streptomyces griseus* 16S rRNA gene clade: use of multi-locus sequence analysis for streptomycete systematics. *Int J Syst Evol Microbiol* 58:149–159
- Gurevich A, Saveliev V, Vyahhi N, Tesler G (2013) QUASt: quality assessment tool for genome assemblies. *Bioinformatics* 29(8):1072–1075
- Güssow HT (1914) The systematic position of the organism of the common potato scab. *Science* 39:431–433
- Jain C, Rodriguez-R LM, Phillippy AM, Konstantinidis KT, Aluru S (2018) High throughput ANI analysis of 90K prokaryotic genomes reveals clear species boundaries. *Nat Commun* 9(1):5114
- Janssen PH (2006) Identifying the dominant soil bacterial taxa in libraries of 16S rRNA and 16S rRNA genes. *Appl Environ Microbiol* 72(3):1719–1728
- Joshi M, Rong X, Moll S, Kers J, Franco C, Loria R (2007) *Streptomyces turgidiscabies* secretes a novel virulence protein, Nec1, which facilitates infection. *Mol Plant Microbe* in 20(6):599–608
- Kers JA, Cameron KD, Joshi MV, Bukhalid RA, Morello JE, Wach MJ, Gibson DM, Loria R (2005) A large, mobile pathogenicity island confers plant pathogenicity on *Streptomyces* species. *Mol Microbiol* 55(4):1025–1033
- Labeda DP, Goodfellow M, Brown R, Ward AC, Lanoot B, Vannanneyt M, Swings J, Kim SB, Liu Z, Chun J, Tamura T (2012) Phylogenetic study of the species within the family Streptomycetaceae. *Antonie Van Leeuwenhoek* 101(1):73–104
- Labeda DP, Dunlap CA, Rong X, Huang Y, Doroghazi JR, Ju KS, Metcalf WW (2017) Phylogenetic relationships in the family Streptomycetaceae using multi-locus sequence analysis. *Antonie Van Leeuwenhoek* 110(4):563–583
- Lambert DH, Loria R (1989) *Streptomyces scabies* sp nov, nom rev. *Int J Syst Evol Microbiol* 39(4):387–392
- Loria R, Davis JR (1989) “*Streptomyces scabies*”. In *Laboratory guide for identification of plant pathogenic bacteria*, 2nd edition, Edited by: Shaad NW pp.114–119. St. Paul: APS Press.
- Loria R, Kers J, Joshi M (2006) Evolution of plant pathogenicity in *Streptomyces*. *Ann Rev Phytopathol* 44:469–487
- Oniki M, Suzui T, Araki T, Sonoda R, Chiba T, Takeda T (1986) Causal agent of russet scab of potato [*Streptomyces* sp.]. *Bulletin of the National Institute of Agro-Environmental Sciences (Japan)*
- Overbeek R, Olson R, Pusch GD, Olsen GJ, Davis JJ, Disz T, Edwards RA, Gerdes S, Parrello B, Shukla M, Vonstein V (2013) The SEED and the rapid annotation of microbial genomes using Subsystems Technology (RAST). *Nucleic Acids Res* 42(D1):D206–D214
- Parks DH, Chuvochina M, Waite DW, Rinke C, Skarshewski A, Chaumeil PA, Hugenholtz P (2018) A proposal for a standardized bacterial taxonomy based on genome phylogeny. *BioRxiv*, p.256800
- Pierzynski GM, Vance GF, Sims JT (2005) *Soils and environmental quality*. CRC Press
- Rodriguez-R LM, Konstantinidis KT (2016) The enveomics collection: a toolbox for specialized analyses of microbial genomes and metagenomes. *PeerJ Preprints* 4:e1900v1 <https://doi.org/10.7287/peerj.preprints.1900v1>
- Roldán-Arjona T, Pérez-Espinosa A, Ruiz-Rubio M (1999) Tomatinase from *Fusarium oxysporum* f. sp. *lycopersici* defines a new class of saponinases. *Mol Plant Microbe* in 12(10):852–861
- SAS Institute, Inc. (1999), *SAS/STAT User’s Guide*, Version 9.4, 1st printing, Volume 2. SAS Institute Inc, SAS Campus Drive, Cary, North Carolina 27513.
- Scholte K, Labruyère RE (1985) Netted scab: a new name for an old disease in Europe. *Potato Res* 28(4):443–448
- Scheible WR, Fry B, Kochevenko A, Schindelasch D, Zimmerli L, Somerville S, Loria R, Somerville CR (2003) An Arabidopsis mutant resistant to thaxtomin A, a cellulose synthesis inhibitor from *Streptomyces* species. *Plant Cell* 15(8):1781–1794
- Shirling ET, Gottlieb D (1966) Methods for characterization of *Streptomyces* species. *Int J Syst Evol Microbiol* 16(3):313–340
- Shirling ET, Gottlieb D (1968) Cooperative description of type cultures of *Streptomyces*. II. Species descriptions from first study. *Int J Syst Bacteriol* 18(2):69–189.
- Sutcliffe IC, Trujillo ME, Goodfellow M (2012) A call to arms for systematists: revitalising the purpose and practises underpinning the description of novel microbial taxa. *Antonie Van Leeuwenhoek* 101(1):13–20
- Thaxter R (1892) *Potato Scab*. Annual Report, the Connecticut Agricultural Experiment Station for 1891–1892:153–160
- Wanner LA (2006) A survey of genetic variation in *Streptomyces* isolates causing potato common scab in the United States. *Phytopathol* 96(12):1363–1371
- Zhang Y, Bignell DR, Zuo R, Fan Q, Huguet-Tapia JC, Ding Y, Loria R (2016) Promiscuous pathogenicity islands and

phylogeny of pathogenic *Streptomyces* spp. Mol Plant Microbe in 29(8):640–650

Zhang Y, Loria R (2017) Emergence of novel pathogenic *Streptomyces* species by site-specific accretion and cis-mobilization of pathogenicity islands. Mol Plant Microbe Interact 30(1):72–82

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