



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

Karima Bouchek-Mechiche, L. Gardan, P. Normand, B. Jouan

► **To cite this version:**

Karima Bouchek-Mechiche, L. Gardan, P. Normand, B. Jouan. 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. *International Journal of Systematic and Evolutionary Microbiology*, 2000, 50 (1), pp.91-99. 10.1099/00207713-50-1-91 . hal-01600668

HAL Id: hal-01600668

<https://hal.science/hal-01600668>

Submitted on 1 Jun 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - ShareAlike 4.0 International License

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

K. Boucek-Mechiche,¹ L. Gardan,² P. Normand³ and B. Jouan¹

Author for correspondence: K. Boucek-Mechiche. Tel: +33 2 99 28 28 10. Fax: +33 2 99 28 51 80.
e-mail: boucek@rennes.inra.fr

¹ INRA, Station de Pathologie Végétale, BP 29 35650 Le Rheu, France

² INRA, Station de Pathologie Végétale et Phytobacteriologie, 49071 Cedex Beaucoz, France

³ UMR 5557 CNRS, Ecologie Microbienne, 69622 Villeurbanne Cedex, France

The genomic relatedness was evaluated by DNA–DNA hybridization for 23 strains (21 were pathogenic and two were saprophytic strains) isolated from lesions of common and netted scab in France and 19 strains from other countries, including type strains of *Streptomyces* species. Three genomospecies were defined within the conventional species of *Streptomyces scabies*, and these genomospecies were different from other pathogenic described species (*Streptomyces acidiscabies*, *Streptomyces caviscabies*) based on previously published phenotypic data. Two of these genomospecies (1 and 3) correspond to new species, for which the names *Streptomyces europaeiscabiei* sp. nov. (with type strain CFBP 4497^T) and *Streptomyces stelliscabiei* sp. nov. (with type strain CFBP 4521^T) are proposed. Genomospecies 2 corresponds to *S. scabies* (with type strain CFBP 4517^T = ATCC 49173^T), and includes only one French strain. The pathogenic strains associated with netted scab lesions constituted a new species that was named *Streptomyces reticuliscabiei* sp. nov. (with type strain CFBP 4531^T). The G+C content of DNA from the three strains CFBP 4497^T (*S. europaeiscabiei*), CFBP 4521^T (*S. stelliscabiei*), CFBP 4531^T (*S. reticuliscabiei*) was 71.3, 71.0 and 69.8 mol%, respectively. Phylogenetic analysis based on 16S rRNA gene sequences showed that the type strain CFBP 4497^T was very similar to the type strain of *S. scabies*, whereas, the type strain of *S. stelliscabiei*, CFBP 4521^T, was very similar to the type strain of *Streptomyces bottropensis*. On the basis of 16S rRNA gene sequences, the type strain of *S. reticuliscabiei*, CFBP 4531^T, differed extensively from the other strains of *Streptomyces* tested.

Keywords: scab, *Streptomyces scabies*, potato, DNA–DNA hybridization, 16S rRNA sequences

INTRODUCTION

Common, netted and russet scabs are diseases of

Abbreviations: ISP, International *Streptomyces* Project; OMPG, 1-o-methyl- α -galactopyranoside.

The EMBL accession numbers for the almost full-length double-stranded 16S rRNA gene sequences are AJ007423 for CFBP 4497^T, AJ007428 for CFBP 4531^T and AJ007429 for CFBP 4521^T.

potato caused by several species of *Streptomyces*. Common scab is prevalent in all potato growing areas of the world. It causes deep- or shallow-pitted lesions on potato tubers and on a number of root crops including carrot, radish, beet and turnip. Netted scab of potato has only been reported in European countries, and causes superficial, brown lesions on the skin of the tubers and on the roots (Labruière, 1971; Bång, 1979; Scholte & Labruière, 1985). Russet scab

has been reported in North America (Harrison, 1962; Faucher *et al.*, 1993) and Japan (Oniki *et al.*, 1986). The symptoms of russet scab on the tuber surface, are very similar to those of netted scab. However, there are two main differences between the two diseases, in the species of *Streptomyces* involved and the optimal conditions for disease development. Common, netted and russet scabs are complex bacterial diseases, because of the diversity of their symptoms and causal agents.

Streptomyces scabies is by far, the most important bacterial species causing common scab. Lambert & Loria (1989a) clarified the taxonomic position of *Streptomyces scabies*, and redefined the species as having grey, smooth spores borne in spiral chains, and as utilizing the nine diagnostic sugars listed in the International *Streptomyces* Project (ISP) (Shirling & Gottlieb, 1966), and producing melanin. *S. scabies* strains form a relatively homogeneous group on the basis of the limited number of phenotypic features studied (Lambert & Loria, 1989a; Faucher *et al.*, 1992). However, molecular studies involving techniques such as DNA–DNA hybridization have shown a great diversity within the strains identified as *S. scabies* (Healy & Lambert, 1991; Paradis *et al.*, 1994). Other species, including *Streptomyces acidiscabies* (Bonde & McIntyre, 1968; Lambert & Loria, 1989b), *Streptomyces caviscabies* (Faucher *et al.*, 1995; Goyer *et al.*, 1996), and other as yet unnamed species of *Streptomyces* (Doering-Saad *et al.*, 1992), also cause common scab. The taxonomic position of strains causing netted scab has not yet been determined. The russet scab strains reported in Canada (Faucher *et al.*, 1993) are related to *Streptomyces aureofaciens*. However, other species of *Streptomyces*, currently unclassified, were also isolated from lesions of russet scab in the United States (Harrison, 1962) and Japan (Oniki *et al.*, 1986).

In France, common and netted scabs cause large economic losses, and identification of the causal agents is needed especially to elaborate efficient strategies to control these diseases. A phenotypic study (Bouček-Mechiche *et al.*, 1998) has shown differences between strains identified as *S. scabies*, resulting in the definition of three phenotypic groups. These groups may correspond to three species of which two are new. Another phenotypic group of strains, different from both *S. scabies* and *S. acidiscabies*, has been associated with symptoms of netted scab.

The aims of this work were (i) to confirm by DNA–DNA hybridization that the new phenotypic groups correspond to new genomic species, (ii) to determine, on the basis of complete 16S rRNA gene sequences, the phylogenetic relationships between the defined species and other species of *Streptomyces* for which 16S rRNA gene sequences have been deposited in sequence databases. According to our results three new species are described: *Streptomyces euro-paeiscabiei* sp. nov., *Streptomyces stelliscabiei* sp. nov. and *Streptomyces reticuliscabiei* sp. nov.

METHODS

Bacterial strains. Forty-two strains were used in this study, 23 of which were isolated in France (21 were pathogenic and two were saprophytic strains) and 19 in other countries (Table 1). All strains were originally isolated from scab symptoms on potato or other root crops. These strains have been phenotypically described and cluster into six phenotypes with some unclustered strains, as indicated in Table 1 (Bouček-Mechiche *et al.*, 1998). All strains were routinely cultured on yeast extract-malt extract agar (Pridham *et al.*, 1956–1957), and were stored at -20°C in tryptone-yeast extract broth (Pridham & Gottlieb, 1948) containing 20% (v/v) glycerol.

DNA extraction and purification. All strains were grown in YEME medium (Chater *et al.*, 1982) for 48 h at 25°C with shaking. The cultures were harvested by centrifugation at 3000 g for 10 min, and the mycelial 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 $^{-1}$) was added and the 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 elsewhere (Brenner *et al.*, 1982).

DNA–DNA hybridization. Native DNA was labelled *in vitro* by nick translation with tritium-labelled nucleotides (Amersham International). The S1 nuclease-trichloroacetic acid method was used for hybridization (Crosa *et al.*, 1973; Grimont *et al.*, 1980). The reassociation temperature was 75°C . DNA–DNA hybridization was achieved using labelled DNAs from CFBP 4497^T (subphenon 1a), *S. scabies* CFBP 4517^T (subphenon 1b), CFBP 4521^T (subphenon 1c), CFBP 4531^T (phenon 2, netted scab strain) and *S. acidiscabies* CFBP 4539^T (phenon 4).

DNA base composition. The G+C contents of CFBP 4497^T, CFBP 4521^T and CFBP 4531^T, were determined by thermal denaturation (Marmur & Doty, 1962) according to the following equation: $G+C \text{ content} = 2.44 (T_m - 53.9)$ (Mandel & Marmur, 1968), where T_m is the melting temperature. *S. scabies* CFBP 4517^T and *S. acidiscabies* CFBP 4539^T were used as standards.

Analysis of the 16S rRNA gene. Double-stranded amplification of the whole *rrs* gene was carried out by a modification of the PCR procedure of Mullis & Faloona (1987). The two following universal primers previously described by Normand *et al.* (1996) were used to amplify the 16S rRNA gene: FGPS5-281 (5'-ATGGARAGYTTGAT-CCTGGCTCA-3', where R stands for G or A and Y for C or T) and FGPS1509'-153 (5'-AAGGAGGGGATCCAG-CCGCA-3'). PCR was carried out in a final volume of 50 µl containing template DNA, reaction buffer (10 mM Tris/HCl, pH 8.3, 1.5 mM MgCl $_2$, 50 mM KCl, 10%, w/v, gelatin), 200 µM each dNTP, 0.5 µM oligomers and 2 U TaqI DNA polymerase (Gibco-BRL). Thirty-five cycles of amplification were carried out: denaturation of DNA at 94°C for 1 min, annealing at 60°C for 1 min and extension at 72°C for 2 min. The amplification products were analysed by subjecting 5 µl of the reaction mixture to electrophoresis in a 2% (w/v) (Metaphor; FMC) agarose gel. A 1500 bp double-stranded DNA fragment was amplified.

Table 1. Origins of strains of *Streptomyces* spp. isolated from various hosts used in this study

Taxon	Phenotypic group*	Host and geographical origin
<i>S. scabies</i> (common scab)		
CFBP 4496, CFBP 4497 ^T , CFBP 4498, CFBP 4499, CFBP 4501, CFBP 4502, CFBP 4506, CFBP 4507, CFBP 4508, CFBP 4509, CFBP 4510	1a	<i>Solanum tuberosum</i> , France
CFBP 4513 (= IPO 1595), CFBP 4511 (= IPO 1626)	1a	<i>Solanum tuberosum</i> , The Netherlands
CFBP 4495	1a	<i>Daucus carota</i> , France
CFBP 4503 (= PD 1030), CFBP 4512 (= PD 1027)	1a	<i>Daucus carota</i> , The Netherlands
CFBP 4504 (= 956C)	1a	<i>Solanum tuberosum</i> , Russia
CFBP 4505 (= PD 259)	1a	<i>Beta vulgaris</i> , The Netherlands
CFBP 4517 ^T (= ATCC 49173 ^T)	1b	<i>Solanum tuberosum</i> , USA
CFBP 4516 (= DNK 043)	1b	<i>Solanum tuberosum</i> , South Africa
CFBP 4518	1b	<i>Solanum tuberosum</i> , France
CFBP 4520 (= EF 35)	1b	<i>Solanum tuberosum</i> , Canada
CFBP 4521 ^T , CFBP 4522, CFBP 4523	1c	<i>Solanum tuberosum</i> , France
CFBP 4524 (= Scab 4017)	Unclustered	<i>Solanum tuberosum</i> , Japan
CFBP 4526 (= DNK 057), CFBP 4528 (= DNK 058)	Unclustered	<i>Solanum tuberosum</i> , South Africa
<i>Streptomyces</i> spp. (netted scab)		
CFBP 4530, CFBP 4531 ^T , CFBP 4532, CFBP 4533, CFBP 4534	2	<i>Solanum tuberosum</i> , France
<i>S. acidiscabies</i> (common scab)		
CFBP 4537 (= 9025), CFBP 4539 ^T (= ATCC 49003 ^T)	4	<i>Solanum tuberosum</i> , USA
CFBP 4538 (= Scab 43)	4	<i>Solanum tuberosum</i> , Japan
<i>S. aureofaciens</i> (russet scab)		
CFBP 4550 (= EF 69)	6	<i>Solanum tuberosum</i> , Canada
CFBP 4551 ^T (= ATCC 10762 ^T)	6	Soil, unknown
<i>S. griseus</i>		
CFBP 4546 ^T (= ATCC 23345 ^T)	Unclustered	Soil, unknown
<i>S. caviscabies</i>		
CFBP 4545 ^T (= EF 87)	Unclustered	<i>Solanum tuberosum</i> , Canada
<i>Streptomyces</i> spp. (saprophytic strains)		
CFBP 4529	Unclustered	<i>Solanum tuberosum</i> , France
CFBP 4543	5	<i>Solanum tuberosum</i> , France

CFBP, Collection Française de Bactéries Phytopathogènes, INRA, Angers, France; ATCC, American Type Culture Collection, Manassas, VA, USA. The numbers in parentheses are the original designation of foreign strains.

* See Bouček-Mechiche *et al.* (1998).

DNA sequencing. DNA was sequenced with an automatic ABI sequencer (Applied Biosystems) by Société ESGS (Evry, France) using four internal primers (FGPS485-292, CAGC-AGCCGCGGTAA; FGPS1047-295, ATGTTGGGTAA-AGTC; FGPS505'-313, GTATTACCGCGGCTGCTG; FGPS910'-270, AGCCTTGCGGCCGTACTCCC), and the amplification primers (Normand *et al.*, 1996). Almost full-length double-stranded 16S rRNA gene sequences were obtained for all strains tested and have been deposited in the EMBL database, with the following accession numbers: AJ007423 for CFBP 4497^T, AJ007428 for CFBP 4531^T and AJ007429 for CFBP 4521^T. GenBank was scanned for related sequences using the BLAST algorithm (Altschul *et al.*, 1997) and the related sequences of representative *Streptomyces* spp. were included in subsequent analyses. Sequences were aligned using CLUSTAL X (Thompson *et al.*, 1997) and indel-containing regions were excluded from the analyses. Matrix pairwise comparisons of nucleic acid sequences were corrected for multiple base substitutions by the two-parameter method of Kimura (1980). Phylogenetic trees were

constructed by the neighbour-joining (Saitou & Nei, 1987), parsimony (Kluge & Farris, 1969) and maximum-likelihood (Felsenstein, 1981) methods. A bootstrap confidence analysis was performed for 1000 replicates to determine the reliability of the distance tree topologies obtained (Felsenstein, 1985). The resulting trees were graphically represented using NJPLOT and PHYLO-WIN software (Perrière & Gouy, 1996).

RESULTS

DNA relatedness

The DNA-DNA hybridization results are shown in Table 2. Thirty-three of the 42 strains tested fell into five discrete DNA relatedness groups (genomospecies). Of the nine other strains, five have not yet been classified, of which three were unclustered (CFBP 4524, CFBP 4526, CFBP 4528) and two were sap-

Table 2. Levels of DNA relatedness between strains of species of *Streptomyces*

Source of unlabelled DNA from:*	Relative binding (%) at 75 °C with labelled DNA from:				
	CFBP 4497 ^T	CFBP 4517 ^T	CFBP 4521 ^T	CFBP 4531 ^T	CFBP 4539 ^T
<i>S. scabies</i> (phenon 1)					
Genomospecies 1 (subphenon 1a)					
CFBP 4495 (F)	90	NT	44	NT	NT
CFBP 4496 (F)	88	NT	NT	NT	NT
CFBP 4497 ^T (F)	100	52	47	19	13
CFBP 4498 (F)	88	42	51	NT	NT
CFBP 4499 (F)	70	50	35	NT	NT
CFBP 4501 (F)	85	47	43	NT	NT
CFBP 4502 (F)	87	50	NT	20	NT
CFBP 4503	77	25	54	NT	NT
CFBP 4504	81	NT	NT	NT	NT
CFBP 4505	70	NT	NT	NT	NT
CFBP 4506 (F)	75	38	46	NT	NT
CFBP 4507 (F)	72	NT	NT	NT	NT
CFBP 4508 (F)	89	42	NT	NT	NT
CFBP 4509 (F)	88	46	NT	NT	NT
CFBP 4510 (F)	96	42	41	25	NT
CFBP 4512	71	37	NT	NT	NT
CFBP 4513	82	49	NT	21	NT
CFBP 4511	78	NT	NT	NT	NT
Mean ± SD	82.9 (9.0)				
Genomospecies 2 (subphenon 1b)					
CFBP 4516	45	95	37	NT	21
CFBP 4517 ^T (= ATCC 49173 ^T)	42	100	40	20	NT
CFBP 4518 (F)	41	72	51	NT	NT
CFBP 4520	42	90	39	NT	NT
Mean ± SD	89 (10.5)				
Genomospecies 3 (subphenon 1c)					
CFBP 4521 ^T (F)	41	52	100	24	17
CFBP 4522 (F)	55	56	85	NT	NT
CFBP 4523 (F)	40	47	95	NT	NT
Mean ± SD	93.3 (7.6)				
Not yet classified (unclustered)					
CFBP 4524	NT	52	36	NT	NT
CFBP 4526	41	45	NT	NT	NT
CFBP 4528	42	48	40	NT	NT
Netted scab strains (phenon 2)					
Genomospecies 4					
CFBP 4530 (F)	19	21	NT	76	NT
CFBP 4531 ^T (F)	20	19	18	100	14
CFBP 4532 (F)	NT	25	18	82	NT
CFBP 4533 (F)	NT	NT	NT	72	NT
CFBP 4534 (F)	NT	NT	NT	73	NT
Mean ± SD	81.7 (13)				
<i>S. acidiscabies</i> (phenon 4)					
Genomospecies 5					
CFBP 4537	NT	NT	NT	NT	85
CFBP 4538	NT	13	NT	14	74
CFBP 4539 ^T (= ATCC 49003 ^T)	15	19	17	16	100
Mean ± SD	86.3 (13)				

Table 2 (cont.)

Source of unlabelled DNA from:*	Relative binding (%) at 75 °C with labelled DNA from:				
	CFBP 4497 ^T	CFBP 4517 ^T	CFBP 4521 ^T	CFBP 4531 ^T	CFBP 4539 ^T
<i>S. aureofaciens</i> (phenon 6)					
CFBP 4550	6	11	NT	9	NT
CFBP 4551 ^T (ATCC 10762 ^T)	5	9	4	7	NT
<i>S. caviscabies</i>					
CFBP 4545 ^T (unclustered)	13	17	16	14	NT
<i>S. griseus</i>					
CFBP 4546 ^T	10	13	10	14	NT
Saprophytic strains					
CFBP 4529 (F) (unclustered)	18	26	NT	24	17
CFBP 4543 (F) (subphenon 5)	25	26	NT	21	19

NT, Strains not tested.

* (F), isolated in France.

rophytic (CFBP 4529, CFBP 4543), and the remaining four strains corresponded to *Streptomyces aureofaciens* (2), *S. caviscabies* (1) and *Streptomyces griseus* (1).

Relatedness within genomospecies was 70–100% homology. Relatedness between the five genomospecies and all other strains tested ranged from 4 to 56% (Table 2).

Genomospecies 1 contained 18 pathogenic strains (subphenon 1a) that were 70–100% (mean = 82.9%; SD = 9) related to pathogenic strain CFBP 4497^T and corresponded to a discrete DNA hybridization group. Twelve of the 18 pathogenic strains were isolated in France, five in the Netherlands and one in Russia, from common scab lesions, mostly on potato, but also on carrot and beet.

Genomospecies 2 contained four pathogenic strains (subphenon 1b) that were 72–100% (mean = 89; SD = 10.5) related to type strain of *S. scabies* and thus belonged to this species. These strains were isolated from common scab lesions on potato. Only one French strain belonged to genomospecies 2, which also contained strains isolated from other countries: one strain was isolated in Canada, one in South Africa and the type strain, *S. scabies* CFBP 4517^T, was isolated in the USA.

Genomospecies 3 contained three pathogenic strains (subphenon 1c) that were 85–100% (mean = 93.3; SD = 7.6) related to pathogenic strain CFBP 4521 and corresponded to a discrete DNA hybridization group. These strains were isolated from star-like lesions on potato in a single region of France.

The three unclustered pathogenic strains, which had the same primary characteristics as *S. scabies* were less than 52% related to labelled DNAs from CFBP 4497^T (genomospecies 1), CFBP 4517^T (genomospecies 2) and CFBP 4521^T (genomospecies 3).

Genomospecies 4 contained five pathogenic strains (phenotypic group 2) that were 72–100% (mean = 81.5; SD = 13) related to CFBP 4531^T and corresponded to a discrete DNA hybridization group. These strains were isolated from netted scab lesions on potato in France.

Genomospecies 5 contained three pathogenic strains of *S. acidiscabies* that were 74–100% (mean = 86.3; SD = 13) related to their type strain, CFBP 4539^T.

DNA base composition

Strain CFBP 4497^T (genomospecies 1) contained 71.3 mol% G + C, CFBP 4521^T (genomospecies 3) had 71.7 mol% G + C and CFBP 4531^T (genomospecies 4) had 69.8 mol% G + C. We obtained G + C content of 70.3 mol% for the reference strains, *S. scabies* CFBP 4517^T and *S. acidiscabies* CFBP 4539^T, which was consistent with the values of 70.5 mol% (CFBP 4517^T) and 71.1 mol% (CFBP 4539^T) determined previously (Healy & Lambert, 1991).

Amplification from chromosomal DNA

Chromosomal DNA isolated from pure cultures of *Streptomyces* isolates was used as a template for amplification. The DNA amplified was, in each case, a single product of the expected length (approx. 1500 bp), corresponding to the region between primers FGPS5-281 and FGPS1509'-153.

Sequence comparison and phylogenetic analysis of the amplified 16S rRNA gene

An almost complete *rrs* (16S rRNA gene) sequence was obtained for all isolates studied. Sequences were compared with those in GenBank and were found to be most similar to those of *Streptomyces* spp. The sequences obtained start at coordinates 20 of the

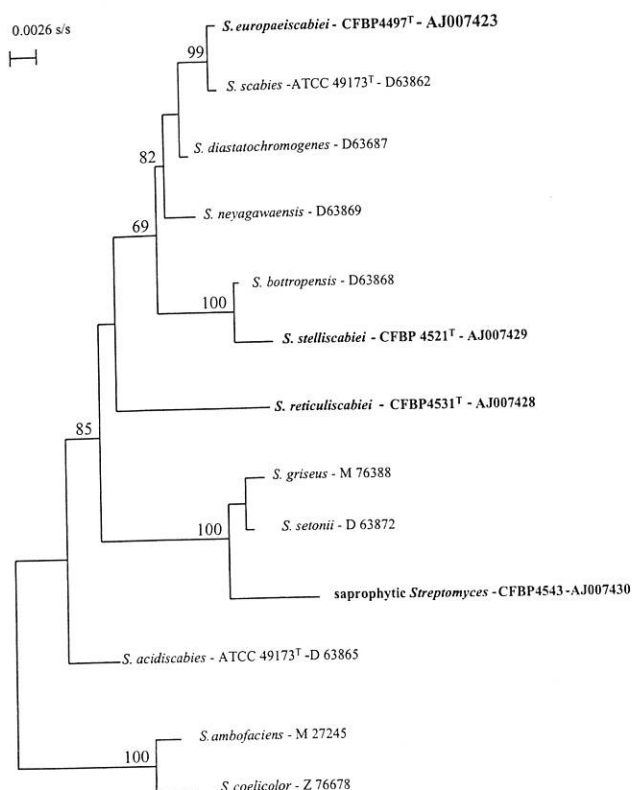


Fig. 1. Phylogenetic tree of *Streptomyces* spp. based on the 16S rRNA gene sequences obtained by the neighbour-joining method (Saitou & Nei, 1987) using a bootstrap approach (Felsenstein, 1985) to determine the reliability of the topology obtained (numbers given above the nodes). The EMBL accession numbers are given after the strain names. The bar indicates a distance of 0.0026 substitutions per site.

S. griseus sequence (EMBL no. M76388) and end at coordinates 1513. Sequences were aligned with published *rrs* sequences from *Streptomyces* spp., and four regions containing indels were identified at positions 57–67, 154–180, 973–977 and 1413–1420. These regions were not included in subsequent analysis.

The levels of similarity among the *Streptomyces* species examined in this study were more than 95.9%. The common scab isolate of genomospecies 1, CFBP 4497^T was most similar to *S. scabies* strains (ATCC 49173^T) with sequence identities of 99.8%. Phylogenetic analysis confirmed the distance findings and grouped CFBP 4497^T in a tight cluster with *S. scabies* ATCC 49173^T sequences, supported by 99% of bootstrap replicates (Fig. 1). The nearest neighbour to the common scab strain, CFBP 4521^T, which belongs to genomospecies 3, was *Streptomyces bottropensis* with 99.6% sequence identity. Clustering of CFBP 4521^T with *S. bottropensis* was supported by 100% of bootstrap replicates and by parsimony (Fig. 1). The level of similarity between the netted scab strain CFBP 4531^T, which belong to genomospecies 4 and the other pathogenic strains of

Streptomyces tested was relatively low. The nearest neighbour to the saprophytic strain CFBP 4543 was *Streptomyces setonii* with 99.2% sequence identity, supported by 100% of bootstrap replicates and by parsimony.

DISCUSSION

In a previous study (Boucek-Mechiche *et al.*, 1998), we showed that all pathogenic strains isolated in France from lesions of common scab on potatoes and other root vegetables were identified as *S. scabies* based on the following primary characteristics used to describe this species: grey spores borne in spiral chains, utilization of the nine ISP sugars (Shirling & Gottlieb, 1966), melanine production and sensitivity to streptomycin. We also demonstrated that *S. scabies* strains are phenotypically heterogeneous, and can be divided into three groups (subphenon 1a, 1b and 1c) and unclustered strains, which were differentiated by the following biochemical test: assimilation of 1-*o*-methyl- α -galactopyranoside (OMPG), *trans*-aconitate, 5-keto-D-gluconate, betain, D(+)trehalose and gentisate. Another phenotypic group (phenon 2) of pathogenic strains was associated with netted scab lesions.

All French strains were tested for pathogenicity on *Solanum tuberosum* cultivars Urgenta (known to be susceptible to common scab) and Bintje (known to be susceptible to both netted or common scabs), seedlings of carrot (*Daucus carota* cv. Premia) or seedlings of radish (*Raphanus sativus* cv. Polka) in a greenhouse at 20 °C (Boucek-Mechiche *et al.*, unpublished).

In this study the DNA relatedness of 23 strains isolated from potato scab lesions in France was assessed and compared with that of 19 strains isolated in other countries, including the type strains of validly described species of *Streptomyces*. DNA–DNA hybridization showed that 21 pathogenic strains of the 23 French strains were grouped into four genomospecies strictly correlated with the phenotypic clusters, while the two saprophytic strains did not fall into these groups.

The 16 pathogenic *S. scabies* strains isolated from common scab lesions in France were phenotypically heterogeneous and formed three genomospecies. Genomospecies 1 and genomospecies 3 were found to constitute new discrete DNA relatedness groups and genomospecies 2 was identified as *S. scabies* because it included the type strain, CFBP 4517^T. Previous studies have shown that the strains identified as *S. scabies* constitute a heterogeneous DNA relatedness group. In fact, Healy & Lambert (1991) showed that the level of DNA relatedness between two strains with the primary characteristics of *S. scabies* could be as low as 20%. Similarly, Paradis *et al.* (1994) found that *S. scabies* includes two diverse genomic groups of strains based on DNA–DNA hybridization, and that these groups also differed in fatty acid and protein profiles. These two studies thus implied that the *S. scabies* species was

too large, and give support to the idea of revising the taxonomic status of these strains.

All strains of genomospecies 1 were distantly related to type strains of *S. scabies* CFBP 4517^T (25–52%) and are distinguished phenotypically by the utilization of *trans*-aconitate, D(+)-trehalose, OMPG and did not use betain. However, phylogenetic analyses based on 16S rRNA gene sequences showed that CFBP 4497^T strain belonging to genomospecies 1 was closely related to the type strain of *S. scabies*, CFBP 4517^T. This conflict between low DNA–DNA hybridization values and high 16S rRNA similarity levels was met by Fox *et al.* (1992) working on *Bacillus* species. Thus, according to Stackebrandt & Goebel (1994), the resolution power of DNA hybridization is significantly higher than the resolution power of sequence analysis of 16S rRNA gene, consequently DNA hybridization remains the optimal method to measure the level of relatedness between highly related organisms.

Strains isolated from hosts other than potato (carrot and beet) were also assigned to genomospecies 1 on the basis of DNA–DNA hybridization. This is consistent with the work of Goyer & Beaulieu (1997), who observed that *Streptomyces* spp. isolated from carrot lesions belong to one of the two genomic clusters of *S. scabies*.

All strains of genomospecies 3 were also distantly related to type strains of *S. scabies* CFBP 4517^T (47–56%) and are distinguished phenotypically by the utilization of *trans*-aconitate, D(+)-trehalose and did not use 5-keto-D-gluconate or betain. The strains of genomospecies 3 were also distantly related to the type strain CFBP 4497^T of genomospecies 1, and can be distinguished phenotypically by the non-utilization of OMPG, 5-keto-D-gluconate or gentisate. However, phylogenetic analysis based on 16S rRNA gene sequences showed that strain CFBP 4521^T of genomospecies 3 was closely related to *S. bottropensis*. Healy & Lambert (1991) also found that some strains of *Streptomyces* sp. phenotypically similar to *S. scabies* and not very similar to type strain CFBP 4517^T on the basis of DNA–DNA hybridization, are very similar to the *S. bottropensis* type strain.

Our phylogenetic analysis, based on 16S rRNA gene sequences, gave results similar to those of Takeuchi *et al.* (1996), who found that all strains with the primary characteristics of *S. scabies* had 16S rRNA gene sequences similar to those of *Streptomyces diastochromogenes*, *S. bottropensis* and *Streptomyces neyagawaensis*, but less similar to those of other species of *Streptomyces*.

All netted scab strains of genomospecies 4 were distantly related (< 25% relatedness) to strains of other scab pathogens: genomospecies 1, genomospecies 2 (*S. scabies*), genomospecies 3, *S. acidiscabies*, *S. caviscabies*, *Streptomyces aureofaciens*. On the basis of phenotypic features (Bouchek-Mechiche *et al.*, 1998), strains of netted scab formed a homogeneous

group readily differentiated by various characteristics (pigmentation, morphology and carbon sources utilization) from other described pathogenic species of *Streptomyces*. These strains were pathogenic only on some susceptible cultivars of potato (data not shown), on which they produced typical symptoms of netted scab on the surface of tubers and on the roots. The 16S rRNA gene sequences, of the strain of genomospecies 4 were not very similar to those of the other strains tested, indicating the lack of a close relationship to other scab pathogens.

The two species, *S. acidiscabies* and *S. caviscabies* were not detected in the regions that were prospected in France. Nevertheless, they may be present, but at a lower frequency than the pathogenic strains of genomospecies 1, 2, 3 and 4 (netted scab strains).

In accordance with the definition of bacterial species of Wayne *et al.* (1987), from our phenotypic and genomic data for the strains isolated from common scab lesions in France, two new species were delineated within the conventional species of *S. scabies*. We suggest the name *Streptomyces europaeiscabiei* for the strains of genomospecies 1 which are European in origin and *Streptomyces stelliscabiei* for the strains of genomospecies 3, isolated in France from star-like lesions on potato. We also identified a new species associated with netted scab lesions in France, for which we propose the name *Streptomyces reticuliscabiei*.

Description of *Streptomyces europaeiscabiei* sp. nov.

Streptomyces europaeiscabiei (eu.ro.pa.ei.sca'bi.ei. L. adj. *europaeus* European; L. n. *scabies* mange; M.L. n. *europaeiscabiei* referring to the European origin of the strains).

Spores are grey and are borne in mature spiral chains. Melanin is produced on tyrosine agar. All ISP sugars are used: L-arabinose, D-fructose, D-glucose, D-mannitol, inositol, raffinose, rhamnose, sucrose, D-xylose. Degradation of xanthine differed between the strains studied. All strains are susceptible to 20 µg streptomycin ml⁻¹ and 0.5 µg crystal violet ml⁻¹. They are not susceptible to 25 µg oleandomycin ml⁻¹ or 10 IU penicillin G ml⁻¹. They utilize *trans*-aconitate, D(+)-trehalose, OMPG, melibiose, 5-keto-D-gluconate and most (about 78%) of the strains assimilate gentisate. They do not use betain, mucate, D-saccharate, DL-lactate and turanose. The G+C content of the type strain is 71.3 mol%. These strains were isolated from common scab lesions, mostly on potato, but also on carrot and beet and have been confirmed to be pathogenic on potato cvs Bintje and Urgenta, on carrot cv. Premia and on radish cv. Polka. The type strain has been deposited in the French Collection of Phytopathogenic Bacteria (Collection Française des Bactéries Phytopathogènes) as CFBP 4497^T, the International Collection of Microorganism from Plants as ICMP 13714^T, and the National Collection of Plant Pathogenic Bacteria as NCPPB 4039^T. This strain was isolated from common scab lesions on potato in

France. It has the physiological and biochemical characteristics of the species.

Description of *Streptomyces stelliscabiei* sp. nov.

Streptomyces stelliscabiei (stel.li.sca'bi.ei. L. n. *stellatus* star; L. n. *scabies* mange; M.L. n. *stelliscabiei* referring to lesions from which these strains were isolated, which look like stars).

Spores are grey and are borne in mature spiral chains. Melanin is produced on tyrosine agar. All ISP sugars are used: L-arabinose, D-fructose, D-glucose, D-mannitol, inositol, raffinose, rhamnose, sucrose, D-xylose. Most strains studied degrade xanthine. All strains are susceptible to 20 µg streptomycin ml⁻¹, 0.5 µg crystal violet ml⁻¹, 100 µg oleandomycin ml⁻¹ and 5 % (w/v) NaCl. They are not susceptible to 25 µg oleandomycin ml⁻¹ or 10 IU penicillin G ml⁻¹. They utilize *trans*-aconitate, D(+)-trehalose, α-D(+)-melibiose but do not assimilate 5-keto-D-gluconate, OMPG, betain, mucate, D-saccharate, DL-lactate, gentisate or turanose. The G + C content of the type strain is 71.7 mol %. These strains were isolated from star-like common scab lesions on potato cv. Belle de Fontenay and have been confirmed to be pathogenic on potato cvs Bintje and Urgenta, on carrot cv. Premia and on radish cv. Polka. The type strain is CFBP 4521^T (= ICMP 13715^T = NCPPB 4040^T). This strain was isolated from star-like common scab lesions on potato tubers cv. Belle de Fontenay in France. This strain has the physiological and biochemical characteristics of the species.

Description of *Streptomyces reticuliscabiei* sp. nov.

Streptomyces reticuliscabiei (re.ti.cu.li.sca'bi.ei. L. n. *reticulum* reticulum; L. n. *scabies* mange; M.L. n. *reticuliscabiei* referring to the reticulum aspect of the symptoms of the disease).

Spores are light grey, and are borne in mature flexuous chains. Melanin is not produced on tyrosine agar. They do not grow in the presence of 0.5 µg crystal violet ml⁻¹, 20 µg streptomycin ml⁻¹, 100 µg oleandomycin ml⁻¹ or 5 % (w/v) NaCl. They are not susceptible to 10 IU penicillin G ml⁻¹ and some (about 60 %) of the strains are not susceptible to 25 µg oleandomycin ml⁻¹. All ISP sugars (fructose, D-glucose, D-mannitol, inositol, raffinose, rhamnose, sucrose, D-xylose) are used as carbon sources. These strains utilize α-D(+)-melibiose, mucate, D-saccharate, D(+)-trehalose, 5-keto-D-gluconate. They do not assimilate *trans*-aconitate or OMPG. Most (about 80 %) of the strains utilize DL-lactate and turanose. Some strains use betain. The G + C content of the type strain is 69.8 mol %. These strains were isolated from netted scab lesions on potato cv. Bintje and have been confirmed to be pathogenic only on potato cv. Bintje. The type strain is CFBP 4531^T (= ICMP 13716^T = NCPPB 4041^T). It was isolated from lesions of netted scab on potato tubers in France.

ACKNOWLEDGEMENTS

We would like to thank J. Marechal (Université Lyon I, France) and S. Belouin (INRA Angers, France) for technical assistance, C. Pasco (INRA Rennes, France), C. Beaulieu (Université Sherbrooke, Québec, Canada), A. de Klerk (Agricultural Research Council, Pretoria, South Africa), R. Loria (Cornell University Ithaca, NY, USA), K. Scholte (Wageningen University, the Netherlands) and C. Bizet (Institut Pasteur, Paris, France) for kindly providing strains. This work received support from GNIS, ONIFLHOR and CNIPT.

REFERENCES

- Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W. & Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* **25**, 3389–3402.
- Bång, H. (1979). Studies on potato russet scab. I. A characterization of different isolates from northern Sweden. *Acta Agric Scand* **29**, 145–150.
- Bonde, M. R. & McIntyre, G. A. (1968). Isolation and biology of a *Streptomyces* sp. causing potato scab in soils below pH 5.0. *Am Potato J* **45**, 273–278.
- Bouček-Mechiche, K., Guérin, C., Jouan, B. & Gardan, L. (1998). *Streptomyces* species isolated from potato scabs in France: numerical analysis of 'Biotype 100' carbon sources assimilation data. *Res Microbiol* **149**, 653–663.
- Brenner, D. J., McWorter, A. C., Leete Knutson, J. K. & Steigerwalt, A. G. (1982). *Escherichia vulneris*: a new species of Enterobacteriaceae associated with human wounds. *J Clin Microbiol* **15**, 1133–1140.
- Chater, K. F., Hopwood, D. A., Kieser, T. & Thompson, C. J. (1982). Gene cloning in *Streptomyces*. *Curr Top Microbiol Immunol* **96**, 69–95.
- Crosa, J. M., Brenner, D. J. & Falkow, S. (1973). Use of single-strand-specific nuclease for analysis of bacterial and plasmid deoxyribonucleic acid homo- and heteroduplexes. *J Bacteriol* **115**, 904–911.
- Doering-Saad, C., Kämpfer, P., Manulis, S., Kritzman, G., Schneider, J., Zakrzewska-Czerminska, J., Schrempfand, H. & Barash, I. (1992). Diversity among *Streptomyces* strains causing potato scab. *Appl Environ Microbiol* **58**, 3932–3940.
- Faucher, E., Savard, T. & Beaulieu, C. (1992). Characterization of actinomycetes isolated from common scab lesions on potato tubers. *Can J Plant Pathol* **14**, 197–202.
- Faucher, E., Otrysko, B., Pradis, E., Hodge, N. C., Stall, R. E. & Beaulieu, C. (1993). Characterization of streptomycetes causing russet scab in Québec. *Plant Dis* **77**, 1217–1220.
- Faucher, E., Pradis, E., Goyer, C., Hodge, N., Hogue, R., Stall, R. E. & Beaulieu, C. (1995). Characterization of streptomycetes causing deep-pitted scab of potato in Québec, Canada. *Int J Syst Bacteriol* **45**, 222–225.
- Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* **17**, 368–376.
- Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.
- Fox, G. E., Wisotzkey, J. D. & Jurtshuk, P., Jr (1992). How close is close: 16S rRNA sequence identity may not be sufficient to guarantee species identity. *Int J Syst Bacteriol* **42**, 166–170.
- Goyer, C. & Beaulieu, C. (1997). Host range of streptomycete strains causing common scab. *Plant Dis* **81**, 901–904.

- Goyer, C., Faucher, E. & Beaulieu, C. (1996). *Streptomyces caviscabies* sp. nov., from deep-pitted lesions in potatoes in Québec. *Int J Syst Bacteriol* **46**, 635–639.
- Grimont, P. A. D., Popoff, M. Y., Grimont, F., Coynault, C. & Lemelin, M. (1980). Reproducibility and correlation study of three deoxyribonucleic acid hybridization procedures. *Curr Microbiol* **4**, 325–330.
- Harrison, M. D. (1962). Potato russet scab, its cause and factors affecting its development. *Am Potato J* **39**, 368–387.
- Healy, F. G. & Lambert, D. H. (1991). Relationships among *Streptomyces* spp. causing potato scab. *Int J Syst Bacteriol* **41**, 479–482.
- Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* **16**, 111–120.
- Kluge, A. G. & Farris, J. S. (1969). Quantitative phyletics and the evolution of anurans. *Syst Zool* **18**, 1–32.
- Labruière, R. E. (1971). *Common Scab and its Control in Seed Potato Crops*. Wageningen, the Netherlands: Centre for Agricultural Publishing and Documentation.
- Lambert, D. H. & Loria, R. (1989a). *Streptomyces scabies* sp. nov., nom. rev. *Int J Syst Bacteriol* **39**, 387–392.
- Lambert, D. H. & Loria, R. (1989b). *Streptomyces acidiscabies* sp. nov. *Int J Syst Bacteriol* **39**, 393–396.
- Mandel, M. & Marmur, J. (1968). Use of ultraviolet absorbance-temperature profile for determining the guanine plus cytosine content of DNA. *Methods Enzymol* **12B**, 195–206.
- Marmur, J. & Doty, P. (1962). Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J Mol Biol* **5**, 109–118.
- Mullis, K. B. & Faloona, F. A. (1987). Specific synthesis of DNA *in vitro* via a polymerase-catalyzed chain reaction. *Methods Enzymol* **155**, 335–350.
- Normand, P., Orso, S., Cournoyer, B., Jeannin, P., Chapelon, C., Dawson, J., Evtushenko, L. & Misra, A. K. (1996). Molecular phylogeny of the genus *Frankia* and related genera and emendation of family *Frankiaceae*. *Int J Syst Bacteriol* **46**, 1–9.
- Oniki, M., Suzui, T., Araki, T., Sonoda, R., Chiba, T. & Takeda, T. (1986). Causal agent of russet scab of potato. *Bull Natl Inst Agro-Environ Sci* **2**, 56–59.
- Paradis, E., Goyer, C., Hodge, N. C., Hogue, R., Stall, R. E. & Beaulieu, C. (1994). Fatty acid and protein profiles of *Streptomyces scabies* strains isolated in eastern Canada. *Int J Syst Bacteriol* **44**, 561–564.
- Perrière, G. & Gouy, M. (1996). WWW-Query: an on-line retrieval system for biological sequence banks. *Biochimie* **78**, 364–369.
- Pridham, T. G. & Gottlieb, D. (1948). The utilization of carbon compounds by some Actinomycetales as an aid for species determination. *J Bacteriol* **56**, 107–114.
- Pridham, T. G., Anderson, P., Foley, C., Lindenfelser, L. A., Hessestine, C. W. & Benedict, R. G. (1956–1957). A selection medium for maintenance and taxonomic study of streptomycetes. *Antibiot Annu* **1956–57**, 947–953.
- Saitou, R. R. & Nei, M. (1987). A neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **44**, 406–425.
- Scholte, K. & Labruière, R. E. (1985). Netted scab: a new name for an old disease in Europe. *Potato Res* **28**, 443–448.
- Shirling, E. B. & Gottlieb, D. (1966). Methods for characterization of *Streptomyces* species. *Int J Syst Bacteriol* **16**, 313–340.
- Stackebrandt, E. & Goebel, B. M. (1994). Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846–849.
- Takeuchi, T., Sawada, H., Tanaka, F. & Madsuda, I. (1996). Phylogenetic analysis of *Streptomyces* spp. causing potato scab based on 16S rRNA sequences. *Int J Syst Bacteriol* **46**, 476–479.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL-X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.
- Wayne, L. G., Brenner, D. J., Colwell, R. R. & 9 other authors (1987). International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.