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Evaluation of the *Sss* AgriStrip rapid diagnostic test for the detection of *Spongospora subterranea* on potato tubers

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Abstract *Spongospora subterranea*, f.sp. *subterranea* (*Sss*), which causes powdery scab, is mainly spread through infected seed tubers and survives in contaminated soil for many years. The visual assessment of tuber lots by inspectors carries the risk of misidentification due to the difficulty of distinguishing lesions caused by either *Sss* or *Streptomyces* spp.. To avoid this, the "*Sss* AgriStrip", a rapid and lab-independent test tool based on a lateral flow immunoassay has been developed, and we assessed its accuracy and sensitivity for detecting *Sss*. The *Sss* AgriStrip performed as well as other lab-based identification methods. The *Sss* AgriStrip, microscopy, ELISA, PCR, and real-time PCR techniques identified infection with *S. subterranea* in all tubers with typical powdery scab lesions. When lots with tubers showing a mixture of typical and atypical (suspicious) symptoms were tested, the presence of *S. subterranea* was confirmed in all lesions by all methods. The DNA content was generally lower in atypical than in typical lesions. Diverse and suspicious symptoms, which were difficult to assign to either powdery or common scab, tested negative with *Sss* AgriStrip and the other methods. This was despite microscopic observation of sporosori-like structures in some samples. Isolation and molecular identification confirmed that these lesions were mostly caused by *Streptomyces* spp. The *Sss* AgriStrip is as sensitive as DAS-ELISA with a detection limit between 1 and 10 sporosori per ml buffer. It is ideal for rapid and selective detection of *Sss* on farms and border inspection points to prevent spread of the pathogen.

Keywords Lateral flow immunoassay · Seed tuber certification · Suspicious lesions · *Streptomyces* spp.

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Introduction

Spongospora subterranea f.sp. *subterranea* (*Sss*), is the plasmodiophorid pathogen that causes powdery scab. This pathogen also acts as a vector of *Potato mop-top virus* (PMTV) (Kirk 2008), one of the causes of "spraing" in potato tubers. Powdery scab is a major problem for potato growers in many areas of the world (Merz 2008). Resting spores of *Sss* can survive in the soil for more than 10 years, and are highly resistant to environmental stresses whilst retaining high and rapid reproductive potential (Merz 2008). Seed tubers infected with *Sss* are the most likely

means of short- and long-distance spread of the pathogen to areas that were previously free from infestation. Since no effective direct control methods are available (Merz and Falloon 2009), prevention of soil contamination by planting clean seed into uncontaminated land is essential.

Most seed certification schemes therefore have a low tolerance for powdery scab infection to minimize the risk of introduction and spread of the disease. However, visual inspection of seed tubers carries the risk of misidentification of scab symptoms, as it is sometimes difficult to distinguish common scab lesions from powdery scab lesions. Common scab, caused by *Streptomyces* spp. is a ubiquitous soil pathogen which is widespread in potato-growing areas of the world. Epidemiological studies on successive potato crops in France (Le Hingrat 1997) and The Netherlands (Turkensteen 1994) showed that the level of common scab on progeny tubers is unrelated to the level of infection of the seed tubers. The tolerance of seed potato tubers is therefore usually higher for common scab compared with powdery scab. The international UNECE standard has a tolerance for certified seeds of 5% of tubers with more than 33.3% of the surface covered with common scab, but only 3% of tubers with more than 10% of the surface covered with powdery scab. Some countries have established more stringent tolerance limits: France tolerates a maximum of 0.2% of contaminated tubers and Switzerland tolerates no more than 1% of tubers with more than five lesions. Regarding their epidemiology, powdery scab and common scab are different in their climatic requirements (temperature and moisture). Common scab is favored by warm, dry and light soils (Lapwood and Adams 1975; Bouchek-Mechiche et al. 2000b), whereas powdery scab prefers cool, wet and heavy soils (Lawrence and McKenzie 1986; van de Graaf et al. 2005). Therefore, irrigation at tuber initiation is an effective means of controlling common scab (Lapwood et al. 1973; Stalham and Friman 1996) but on the other hand favours powdery scab (de Boer 2000; Wale 2000b).

The differences in the biology and epidemiology underline the importance of accurate detection of both pathogens and diagnosis of both diseases. Several sensitive and specific methods are available for the detection of *S*ss on tubers. These include PCR (Bulman and Marshall 1998; Bell et al. 1999), ELISA with monoclonal antiserum (Merz et al. 2005), and real-time PCR (van de Graaf et al. 2003; Bouchek-Mechiche et al. 2004). Although these diagnostic tools are very useful, they require technical knowledge, are time consuming, and need specific protocols and laboratory equipment. This makes them inappropriate for routine detection of the pathogen during the certification process where inspection of potato lots for blemish diseases is done at the place of production or storage or at border inspection points. These tools are also not adapted for large-scale scoring of potato tubers during field experiments where the two pathogens may survive in the same soil and could produce suspicious symptoms on tubers. For these specific uses (potato seed and ware inspection as well as field scoring), there is a need for a quick and easy test to check dubious symptoms and avoid the risk of misidentification between the two diseases.

The same monoclonal antibodies developed for specific detection of *Sss* in DAS-ELISA by Merz et al. (2005), have been made commercially available in a lateral flow immunoassay format (*Sss* AgriStrip) for rapid on-site detection of the pathogen. The *Sss* AgriStrip could be an appropriate tool for routine identification of powdery scab symptoms on tubers if its specificity and sensitivity are equivalent to those of other validated methods. We therefore aimed to compare the accuracy and sensitivity of the *Sss* AgriStrip with DAS-ELISA, PCR, real-time PCR, and microscopy in the routine diagnostis of *Sss* using tubers showing different types of scab symptoms (typical and atypical=suspicious lesions).

Materials and methods

General methodology

AgriStrip

Operational description The *Sss* AgriStrip is a one-step assay which has been developed and manufactured by BIOREBA AG, Reinach, Switzerland. The assay is based on lateral flow immunochromatography using monoclonal antibodies which are specific to resting spores of *Sss* (Merz et al. 2005). The specific antibodies are immobilised on cardboard strips (Fig. 1) on the test line of the membrane and are conjugated to colloidal gold particles on the lower

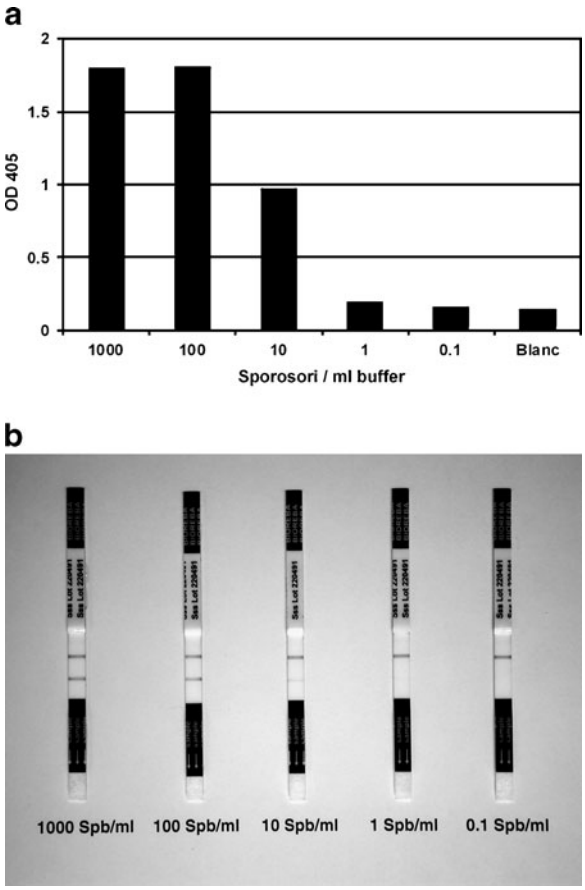


Fig. 1 Comparison of the sensitivity of DAS-ELISA (**a**) and the *Sss* AgriStrip (**b**) with a dilution series of *Spongospora subterranea* sporosori in buffer

part of each test strip. Once a strip is inserted into a sample extract, the liquid migrates upwards and dissolves the antibody-gold conjugate. Any antigen present in the extract binds to this conjugate forming an antigen-antibody-gold complex, which is then captured by the immobilized antibody on the membrane. Gold antibody conjugate without antigen does not bind to the test line and is subsequently captured by the control line. Both test and control lines become visible with positive extracts (containing the antigen), whereas negative samples produce the upper control band only. Bands start developing after 1–2 min and reach maximum intensity after 10–15 min.

Test protocol Peel cuttings from potato tubers containing scab symptoms were homogenized for 1 min in 4 ml of extraction buffer (BIOREBA AG) in a special extraction bag ('Universal', BIOREBA AG) using the hand model homogenizer (BIOREBA AG). A few drops (about 150 µl) of the homogenate were then transferred to a cuvette and the strip was applied to this.

Microscopic observation

The contents of scab lesions were scrapped from tubers. The resulting powder was placed on microscope slides, stained with methyl blue in lactic acid (1 g methyl bleu, 600 ml lactic acid), and observed with light microscopy at x100 and x400 magnification.

ELISA test

The DAS-ELISA was performed using a monoclonal antibody developed against the sporosori of *Sss* (Merz et al. 2005) following the manufacturer's instructions (BIOREBA AG).

PCR methods

DNA extraction The soil DNA extraction method of Bell et al. (1999) was used with some modifications. For each sample, tuber skin with scab lesions (0.2 g) was removed and transferred into 2-ml screw cap tube containing 0.1 g of 1 mm glass beads and 1 ml of extraction buffer [2% hexadecyltrimethyl ammonium bromide (CTAB), 1.5 M NaCL, 120 mM Na2HPO4, pH 8.0]. Each tube was vortexed for 1 min, sonicated in a water bath for 30 min, and then shaken at maximal speed in a Bio 101 Bead Beater for 1 min to disrupt sporosori. Solid debris was pelleted by centrifugation for 10 min at 6000 rpm at 10°C and the supernatant (approximately 700 µl) was transferred to a fresh microtube. DNA was purified by Sephadex spin columns as described by Bell et al. (1999). The extracted DNA was used in standard PCR and real-time PCR.

Standard PCR PCR amplification was performed using *Sss* specific primers *Sps1* and *Sps2* which were previously developed by Bell et al. (1999). The final reaction mixture (25 µl) contained 160 µM of each dNTPs (Eurobio), 2.5 µl (10X) reaction buffer (Promega), 1 U Taq polymerase (Promega), 0.3 µM of each primer (Sigma Genosys), and 5 µl of 1/10 to 1/50 diluted DNA (representing 10 to 50 ng). The PCR amplification was performed in a Tetrad cycler

(MJ Research) using thermal cycling conditions described in Bell et al. (1999). PCR products were analysed by electrophoresis on 1% agarose gels (Eurobio) in 1X TBE buffer (89 mM Tris base, 89 mM boric acid, 2 mM EDTA, pH8.0), stained with Syber safe (Invitrogene) and photographed under UV illumination. *Sps 1* and *Sps 2* were designed to yield an amplification product of 391 bp.

Real-time PCR (TaqMan™) Primers and probes for Real-time PCR (TaqMan™) were designed and tested for their sensitivity and specificity in a previous study as described below.

The internal transcribed spacer regions (ITS1 and ITS2) of *S. subterranea* were accessed from the GeneBank and EMBL databases. The software primer Express® (Applied Biosystems) was used to design forward SS1 (5'-ACC TGG GTG CGA TTG TCTG-3') and reverse SS2 (5'-TGC ATA AGG TTT CGA GCT AGCC-3') primers as well as the SSPr TaqMan® probe (5'-TGA AGG GTG ACG CCC GCT CTG-3'). The fluorogenic SSPr probe was labelled at the 5' end with the fluorescent FAM reporter dye (6-carboxy-fluorescein) and modified at the 3' end with the TAMRA quencher dye (6-carboxy-tetramethylrhodamine). The specificity of the designed primer and probe sequences was confirmed before synthesis following a database (BLAST and FASTA programs) search of DNA sequences. Likewise, after synthesis, the sensitivity and the specificity of these primers and probe were tested. Their sensitivity was similar to that of van de Graaf et al. (2003) and they were unable to amplify DNA from a range of potato pathogens tested including *Streptomyces* spp.(data not shown).

Real-time PCR amplification The reaction components and thermal cycle protocol used in this study were carried out as described by van de Graaf et al. (2003). A standard dilution series of target DNA was used to create standard curve. C_T values from standards containing known amounts of DNA were used to calculate the quantity of DNA in the unknown samples. Results were expressed as pg DNA/µl.

Comparison of the sensitivity of the *Sss* AgriStrip with DAS-ELISA

Sporosori were scraped out of lesions of infected tubers of cv. Agria, sieved (100 µm mesh size) and suspended in a small amount of double distilled water. The concentration of sporosori was determined using a haemacytometer. Aliquots were used to produce a dilution series with 1000, 100, 10, 1 and 0.1 sporosori/ml either in *Sss* AgriStrip extraction buffer (BIOREBA AG) or in extraction buffer 'General' (BIOREBA AG), respectively, for *Sss* AgriStrip and ELISA tests. For ELISA, the samples were homogenized by adding 1 g of a sand mixture (two fractions of particle sizes 0.7–1.2 mm and 1.5–2.2 mm; Carlo Bernasconi AG, Baerschwil, Switzerland) and shaking them at 25 ^{0}C in the dark in a 22 ml glass flask with a screw cap overnight (linear motion, 200 rpm, 16 h; LSR, Adolf Kuehner AG, Birsfelden, Switzerland). After centrifugation at 2000 rpm for 5 min (Allegra X-12R, Beckmann Coulter, Fullerton, USA), 200 µl of the supernatant was used in DAS-ELISA which was performed using a monoclonal antibody following the manufacturer's instructions (BIOREBA AG). The absorbance was recorded at 405 nm after 60 min substrate incubation.

The samples for assessment using the *Sss* AgriStrip dilution series (4 ml each) were further processed as described above.

Comparison of the detection ability of the *Sss* AgriStrip with microscopy, DAS-ELISA PCR and real-time PCR

Samples with scab-like symptoms

Nineteen lots of six to 10 tubers each from 15 potato varieties with different types of scab symptoms were collected during potato inspection. Tubers were washed with tap water, photographed and then separated in four groups: 1) four lots of tubers with typical powdery scab lesions only, 2) three lots of tubers with typical and atypical (suspicious) symptoms, 3) ten lots of tubers with only atypical symptoms, and 4) two lots of tubers with typical common scab lesions. All lots were analysed with detection and quantification tools (*Sss* AgriStrip, microscopy, DAS-ELISA, PCR, real-time PCR) as described above.

Sample preparation For each lot, replicates of 0.2 g of tuber skin with scab lesions were removed from tubers and kept in Eppendorff tubes until they were analyzed by the different methods. For each test, samples were analyzed in duplicate and all of the

tests were repeated a second time with the same samples.

Search for the presence of *Streptomyces* spp. in samples detected free of *S. subterranea*

Streptomyces spp. isolation

Atypical lesions from scabby tubers where *Sss* could not be detected were cut off and the *Streptomyces* spp. isolation method as described by Bouchek-Mechiche et al. (1998) was applied. Saprophytic *Streptomyces* spp. may be isolated together with pathogenic strains from common scab lesions and it is not possible to differentiate them on the basis of colony morphology or on biochemical characteristics. Therefore, a set of two to four separated colonies was selected randomly per isolation to avoid the risk of selecting only saprophytic isolates. *Streptomyces* isolated from lot 13 was not included in this study. A few selected isolates, belonging to lots 8 and 16 were contaminated by other bacteria during the study, and then excluded for the analyses. For these lots only 1 isolate was tested (Table 2). The isolates were stored at 4°C in tubes containing Potato Dextrose Agar (PDA) until DNA extraction and amplification and pathogenicity testing.

DNA extraction and amplification

DNA extraction was performed as described by Bouchek-Mechiche et al. (2000a). The suitability of DNA preparations for PCR amplification was verified by PCR amplification of 16 S rRNA genes using the universal primers pairs, 16 s 1 F and 16 s 1R (Bukhalid et al. 2002).

PCR analysis of genes encoding thaxtomin synthetase (txtAB)

The presence or the absence of the *txt*AB genes encoding the pathogenicity determinant thaxtomin in *Streptomyces* spp. was determined by PCR as described by (Wanner 2006) using the primers *Txt*AB1 and *Txt*AB2.

Pathogenicity testing

Pathogenicity of isolated *Streptomyces* spp. was assessed on potato cv. Bintje and on radish cv. Polka (both very susceptible to common scab) in the growth chamber at 22°C and 80% relative humidity with a 16 h photoperiod. Artificial soil inoculation was performed as described by Wanner (2004). Potato-tuber scoring was performed as described by Bouchek-Mechiche et al. (2000b), and radish disease scoring was performed as described by Wanner (2004). Six pots per isolate and host were planted. *Streptomyces europaeiscabiei* CFBP 4501, inducing common scab (Bouchek-Mechiche et al. 2000a; b) was used as a positive control, and non-inoculated soil as a negative control.

Results

Comparison of the sensitivity of the *Sss* AgriStrip with DAS-ELISA

All tests with both methods gave strongly positive results at concentrations of 1000 and 100 sporosori/ml, showing no difference in the optical density and the intensity of the test line (Fig. 1). The antiserum also detected 10 sporosori per ml buffer but the A_{405} value was reduced to about 50% in ELISA and the test line of the *Sss* AgriStrip was weak. No reaction was obtained with the *Sss* AgriStrip when the concentration of 1 sporosorus per ml buffer was tested and ELISA gave an A_{405} value just slightly above the absorbance of blank wells (0.19 and 0.14, respectively). Neither method was able to detect 0.1 sporosorus per ml buffer.

Comparison of the detection ability of the *Sss* AgriStrip with microscopy, DAS-ELISA, PCR and real-time PCR

All lots of tubers with typical powdery scab symptoms (group 1) tested positive for the presence of *Sss* with the *Sss* AgriStrip as well as with the other methods. Real-time PCR revealed different quantities of DNA between lots (Table 1) and also between replicates of the same lot (data not shown). It was observed that on the same tuber, some of the lesions were full of visible powdery mass (sporosori) and others were partially empty.

Group 2 included three lots containing tubers with typical and atypical powdery scab symptoms, sometimes together on the same tuber. The atypical

Table 1 Detection of *Sss* on different potato tubers lots with typical or atypical powdery scab or common scab lesions, using *Sss* AgriStrip, microcopy, ELISA, PCR or real-time PCR. + and—indicate positive and negative detection

Group	Lots	Symptoms	*Sss* AgriStrip	Microscopy	ELISA	PCR	qPCR	pg DNA/µl[1]
1	Lot 1	TPL	+	+	+	+	+	6300
	Lot 2	TPL	+	+	+	+	+	700
	Lot 3	TPL	+	+	+	+	+	2600
	Lot 4	TPL	+	+	+	+	+	1410
2	Lot 5a	TPL	+	+	+	+	+	1120
	Lot 5b	ATL	+	+	+	+	+	242
	Lot 6a	TPL	+	+	+	+	+	150
	Lot 6b	ATL	+	+	+	+	+	52
	Lot 7a	TPL	+	+	+	+	+	176
	Lot 7b	ATL	+	+	+	+	+	58
3	Lot 8	ATL	−	−	−	−	−	0
	Lot 9	ATL	−	−	−	−	−	0
	Lot 10	ATL	−	(+)	−	−	−	0
	Lot 11	ATL	−	−	−	−	−	0
	Lot 12	ATL	−	−	−	−	−	0
	Lot 13	ATL	−	−	−	−	−	0
	Lot 14	ATL	−	−	−	−	−	0
	Lot 15	ATL	−	−	−	−	−	0
	Lot 16	ATL	−	−	−	−	−	0
	Lot 17	ATL	−	(+)	−	−	−	0
4	Lot 18	TCL	−	−	−	−	−	0
	Lot 19	TCL	−	−	−	−	−	0

[1] mean data from two replicates

TPL: typical powdery scab lesions

ATL: atypical lesions (suspicious)

TCL: typical common scab lesions

(+): few doubtful sporosorus−like bodies

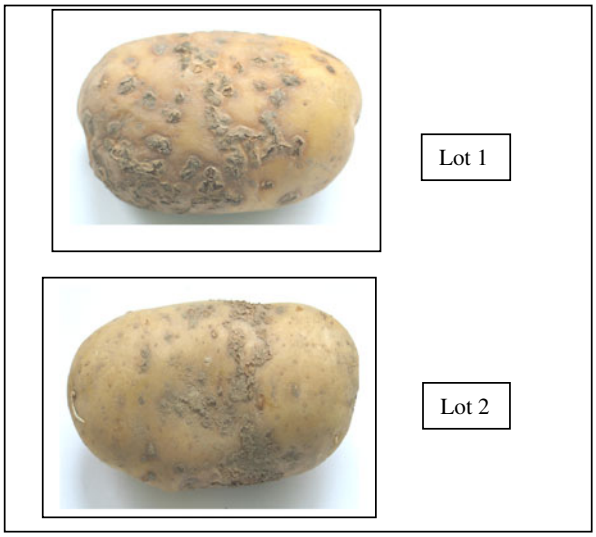
lesions observed on the three lots were similar in appearance. These lesions were diffuse under the periderm (Fig. 2c, lot 5b) and contained no visible powdery mass of dark brown sporosori, which are characteristic of powdery scab. All group 2 samples (typical and atypical) tested positive for the presence of *Sss* with each method. On average, the quantity of DNA present in samples with atypical lesions was lower than that in typical lesions from the same lot, e.g. for lot 5, the amount of DNA was 242 pg/µl in sample 5b (with atypical lesions), and 1120 pg/µl in sample 5a (with typical lesions) (Table 1). As in group 1, real-time PCR revealed different quantities of DNA between samples with the same types of symptoms.

Group 3 included lots with only atypical symptoms which were difficult to assign to powdery scab or common scab. The appearance of the symptoms in the lots was very diverse (Fig. 2d). All symptoms tested negative for the presence of *Sss* with the *Sss* AgriStrip as well as with the other methods, although a few sporosorus-like bodies were observed in atypical lesions removed from tubers of two lots (lot 10 and lot 17).

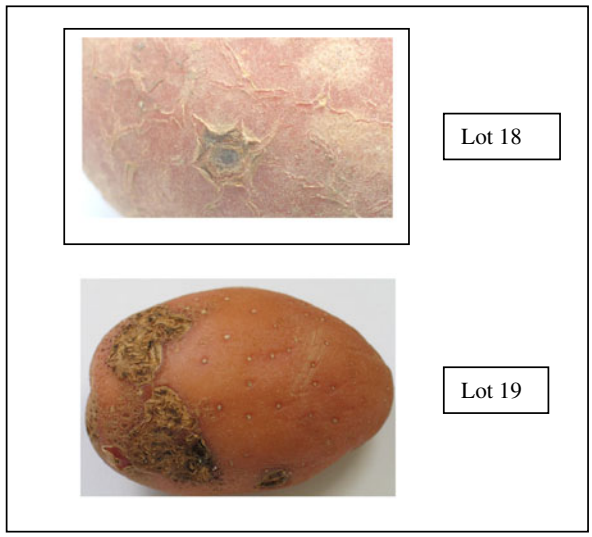
The two lots of group 4 all had typical common scab symptoms (Fig. 2b). Lot 19 consisted of tubers grown in soil which was artificially inoculated with *S. europeascabiei*. Both lots tested negative for the presence of *Sss*.

Search for the presence of *Streptomyces* spp. in samples detected free of *S. subterranea*

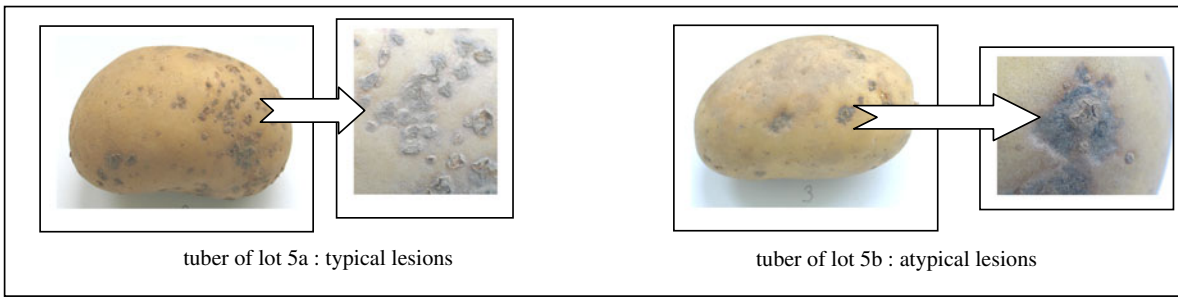
Pathogenic *Streptomyces* spp. were isolated from most of the atypical lesions negative to the presence of *Sss* (Table 2). DNA was successfully extracted from the 22 selected isolates and the 16 S rDNA gene was PCR amplified using universal primers 16S-1 F and 16S-1R (Table 2), indicating the suitability of the DNA preparations for PCR amplification. Assessment of the presence or absence of the txtAB genes by PCR showed that 18 of the 22 selected isolates were txtAB-positive and four were negative.



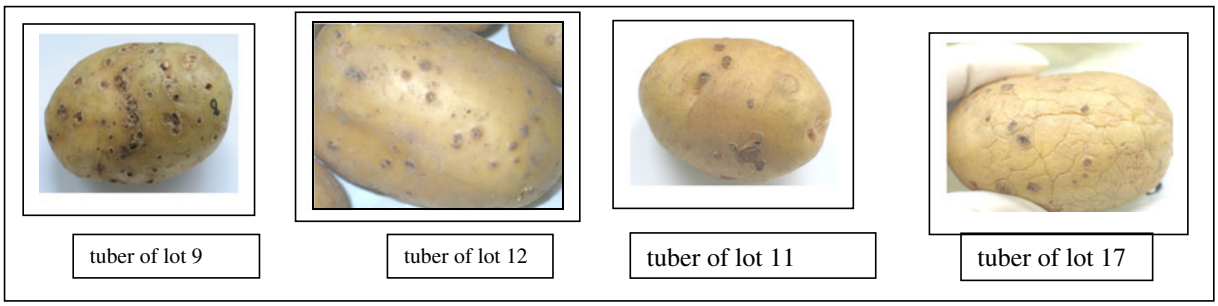
a Tubers with typical lesions of powdery scab (group 1)



b Tubers with typical lesions of common scab (group 4)



c Tubers with typical and atypical lesions(group 2)



d Tubers with atypical lesions (group 3)

Fig. 2 A set of tubers with typical and atypical symptoms examined in this study: **a** Tubers with typical lesions of powdery scab (group 1) **b** Tubers with typical lesions of common scab (group 4) **c** Tubers with typical and atypical lesions (group 2) **d** Tubers with atypical lesions (group 3)

The correlation between *txt*AB and pathogenicity was confirmed in all of the isolates on potato cv. Bintje, and on radish cv. Polka (Fig. 3). All *txt*AB-positive isolates were very pathogenic with the scab lesion severity ranging from 3.6 to 4.6 (on 0 to 5 scale) on radish and 66 to 100% (on 0 to 100% scab index) on potato (Table 2). All of the *txt*AB-negative isolates were non-pathogenic on radish (scab index

Table 2 Evaluation of pathogenicity to potato and radish plants, and for the presence of the *txtAB* operon, for *Streptomyces* spp. isolates taken from atypical potato tuber lesions negative for the presence of *Sss*

Lot	Isolate[a]	Radish scab score[b]	Scab index on potato (%)[c]	pathogenicity[f]	*Streptomyces* 16 S rDNA[g]	*Streptomyces txtAB genes*[h]
8	8a	4.57[d]±0.25[e]	66,56[d]±9[e]	+	+	+
9	9a	4.38±0.25	100±0	+	+	+
	9b	4.25±0.29	84±7.8	+	+	+
10	10a	3.98±0.54	92±6.7	+	+	+
	10b	3.63±0.42	97±4.5	+	+	+
	10c	4.21±0.26	83±10	+	+	+
	10 d	3.88±0.5	100±0	+	+	+
11	11a	4.56±0.23	92±6.5	+	+	+
	11b	0.00	0.00	−	+	−
	11c	4.35±0.55	91±15	+	+	+
12	12a	3.96±0.33	92.6±1.3	+	+	+
	12b	4.32±0.15	95.5±5	+	+	+
	12c	4.00±0.57	98.5±2.3	+	+	+
	12 d	4.5±0.00	84±14	+	+	+
13	13a	NT [i]	NT	NT	NT	NT
	13b	NT	NT	NT	NT	NT
	13 d	NT	NT	NT	NT	NT
14	14a	4.11±0.5	99±2	+	+	+
	14b	4.28±0.19	95.5±4	+	+	++
	14c	4.47±0.06	99±1.65	+	+	+
15	15a	4.40±0.11	96±3.50	+	+	+
	15b	4.14±0.14	94±5.09	+	+	+
16	16a	0.00	2.2±3.8	−	+	−
17	17a	0.05±0.08	3.7±3.9	−	+	−
	17b	0.2±0.11	1.5±2.5	−	+	−
Pos. control	CFBP 4501 [j]	4.46±0.2	73±6.5	+	+	+

[a] in each lot, two to four colonies were selected, except for lot 8 and 16 where two isolates were contaminated and excluded for the study

[b] radish scoring data on a scale of 0 to 5; 0=no lesion, 1=discrete superficial lesions, less than 10 mm in diameter, 2=coalescing superficial lesions, more than 10 mm in diameter, 3=raised lesions, less than 10 mm in diameter, 4=coalescing raised lesions, more than 10 mm in diameter, and 5=pitted or sunken lesions

[c] scab index calculated according to the formula SI=LS X SE x 100/15 where LS is the lesion severity score, rated on 1–3 scale and SE is the symptom extension rated on a 0–5 scale (Bouchek-Mechiche et al. 2000b)

[d] mean of six replicates

[e] standard deviation

[f] isolates were considered pathogenic on radish (scab index above treshold of pathogenicity of 1.0) and on potato (scab index>5%)

[g] PCR amplification of the 16 s rDNA by universal primers 16 s 1 F and 16 s 1R (Bukhalid et al. 2002)

[h] Presence or absence of the thaxtomin biosynthesis operon *txt AB* as determined by PCR

[i] Not tested

[j] *Streptomyces europaeiscabiei* CFBP 4501 is available in the French collection of phytopathogenic bacteria (CFBP); it's inoculum was produced as *Streptomyces* spp. isolated in this study

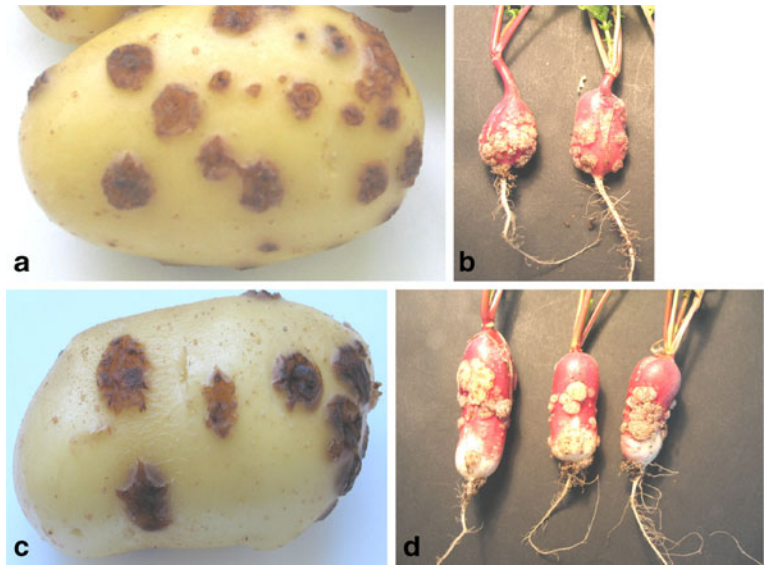


Fig. 3 Examples of symptoms induced on potato cv. Bintje and on radish cv. Polka inoculated with *Streptomyces* spp. in growth chamber pathogenicity test: **a** and **b** symptoms induced by *S. europaeiscabiei* CFBP 4501, **c** and **d** symptoms induced by *Streptomyces* isolated from lot 12

below threshold of pathogenicity of 1.0) and on potato (scab index <5%).

Discussion

The inspection of potato seed and ware lots for the presence of blemish diseases is visual and sometimes even performed on unwashed tubers. Precision of diagnosis in this assessment is a serious problem for the inspectors because *Streptomyces* species may cause similar symptoms to those of *Sss*- caused powdery scab. Difficulties in distinguishing the symptoms of the two different diseases leads to negative consequences either in disseminating *Sss* in soils free from the presence of the pathogen, or rejecting lots with tolerable levels of common scab. These difficulties in identification are also a real problem for scoring potatoes in field trials for disease resistance screening or epidemiological studies.

Using PCR and real-time PCR, Bell et al. (1999), Bouchek-Mechiche et al. (2004) and van de Graaf et al. (2005) found that these methods were able to detect *Sss* inoculum even on symptomless tubers. However, these tools need specific protocols, equipment, time, and technical expertise. Microscopic observation may be an easier way to detect *Sss* in a lesion, but recognition of the crucial structures requires considerable experience. Sporosori vary in size (19–85 μm in diameter), and in shape (spherical, ovoid, polyhedral or irregular), and this can make identification difficult.

The monoclonal antibody that was highly specific for *Sss* sporosori detection (Merz et al. 2005) was first used in a DAS-ELISA format for large-scale screening in the laboratory. This is now commercially available as the *Sss* AgriStrip, an easy and fast test for use in the field. Tubers of 19 different lots with typical and atypical (suspicious) symptoms were analyzed to investigate the reliability and performance of the *Sss* AgriStrip test in comparison with the results of well-established, highly sensitive and specific laboratory methods. Results from the *Sss* AgriStrip were highly consistent with these other techniques. *Sss* was detected in all tubers with typical symptoms but only in three out of the 13 lots with atypical lesions. The atypical symptoms of the three samples belonged to the lots which also possessed tubers with typical lesions. The appearance of the atypical lesions was similar for the three lots, presenting diffuse brown necrotic tissue under the tuber periderms and unrelated to the cultivars. These diffuse symptoms with no visible powdery mass could contain immature sporosori in the lesions which may explain the low quantity of DNA found in these samples.

Considerable variation was noticed within the DNA quantity extracted from similar lesions of the same lot (data not shown) and between lots. This may be explained by the fact that, even on the same tuber, some of the lesions were full of sporosori and others

were partly empty. DNA quantity could also be influenced by the variation in size of the sporosori. Variation was also observed by De Haan and Van Den Bovenkamp (2005) in the real-time PCR Ct-values obtained from the same number of sporosori from individual seed lots.

The cause of atypical (suspicious) symptoms, which tested negative for the presence of *Sss,* could be assigned to *Streptomyces* spp. through reproduction of common scab symptoms in the growth chamber pathogenicity test and by amplifying the *txt*AB genes, the determinant of pathogenicity in *Streptomyces* species. These results agree with those of De Haan and Van Den Bovenkamp (2005), who showed that most of the suspicious symptoms analyzed by ELISA and real-time PCR were not caused by *Sss* infections. The authors assumed but did not verify that these symptoms were due to *Streptomyces* spp. In the present study, we demonstrated the origin of the suspicious symptoms using specific tools for detection and identification of both *Sss* and *Streptomyces* spp. We also demonstrated that the *Sss* AgriStrip can detect the presence of *Sss* in the lesions with a detection limit similar to the DAS-ELISA laboratory method.

Microscopic observation of some of the atypical symptoms that tested negative for the presence of *Sss* revealed a few sporosorus-like structures. De Haan and Van Den Bovenkamp (2005) reported that the microscopy test overestimated the presence of *Sss* in 32 out of 118 samples compared with ELISA and real-time PCR. It is likely that some sponge-like structures may be confused with sporosori and therefore complicate the microscopic examination.

Some of the *Streptomyces* spp. isolated from tubers with atypical lesions (lot 11, 16 and 17) neither induced symptoms on tested plants, nor possessed the *txt*AB genes. This might be due to the saprophytic isolates which were found together with the pathogenic isolates in the same lesions and selected for the pathogenicity test. The traditional detection of pathogenic *Streptomyces* spp. based on isolation and pathogenicity testing is laborious and time consuming and has the risk of selecting saprophytic isolates. It could be simplified by direct DNA extraction from the lesions and PCR amplification of the *txt*AB marker. Our data were in agreement with those of Wanner (2006) who obtained a high correlation between PCR amplification of *txt*AB genes and the results of greenhouse pathogenicity tests.

The present study demonstrates the simplicity, robustness and sensitivity of the *Sss* AgriStrip, which makes it ideally suited for rapid detection of *Sss* on farms and at border-inspection points. This test will substantially increases the accuracy of inspection procedures and field scoring based only on visual assessment. In addition, the *Sss* AgriStrip will be of considerable value in training new plant health inspectors in disease recognition.

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