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Diversity of microorganisms associated with atypical superficial blemishes of potato tubers and pathogenicity assessment

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Abstract Skin blemishes of potato (*Solanum tuberosum* L.) tubers can cause severe economical losses to production. Some blemishes are due to known pathogens and others whose causes are unknown are called atypical blemishes. The present work aims at determining the origin of superficial atypical blemishes on a set of 204 tubers coming from 12 different French regions producing potato. The diversity of fungi and *Streptomyces* bacteria associated with blemishes was investigated by systematic isolation followed by identification by sequencing the internal transcribed spacer of the ribosomal DNA for fungi and by sequencing the 16S ribosomal DNA for bacteria. We found a high microbial diversity represented by 349 fungal isolates belonging to at least 47 different species and 21 bacterial strains of *Streptomyces* sp. The most represented fungi belonged to the genera *Fusarium*, *Rhizoctonia*, *Alternaria*, *Penicillium*, and *Clonostachys*. The pathogenicity of representative isolates was assessed in three bioassays; two bioassays based on single inoculations in previously sterilized potting mixture, and one bioassay based on both single and double inoculations under hydroponic conditions. We fulfilled the Koch's postulates for *Rhizoctonia solani* AG 3 producing sclerotia. For other fungal and bacterial strains, our results did not show any causality or relationship between a single isolate or a complex and the occurrence of the blemishes. Moreover, the observation of irregular polygonal sunken corky lesions (polygonal lesions)—the most frequent atypical blemish—on non-inoculated tubers, suggested that the atypical blemishes could as well be a reaction of the plant to stressful environmental conditions.

Keywords Koch's postulates · Pathogenicity tests · *Solanum tuberosum* · Sequencing · *Rhizoctonia solani* · *Streptomyces*

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Introduction

Potato (*Solanum tuberosum* L.) is the fourth main crop in the world after wheat, rice, and maize. Since the early 1990s, the potato sector has undergone major changes worldwide. The global production increased

by 20% especially in developing countries which are changing their nutritional habits particularly in urban areas (Lutaladio and Castaidi 2009). In most European countries, ware potatoes are now washed before sale. Washing the tubers reveals superficial blemishes and may reduce the commercial value of the commodity. Blemishes or superficial alterations affect only the tuber skin, without affecting the taste or the nutritional properties. However, they have a negative cosmetic effect on the tubers and destroy the integrity of the natural barrier of the skin, forming an entry point for pathogenic microorganisms. Moreover, it has been shown that skin visual appearance is the most important factor influencing consumer behaviour in fresh potato purchase. Economical data about such potential losses are difficult to estimate but all potato sectors, i.e. seed, ware, and processing are affected.

Potato tubers can show a large range of superficial blemishes. These blemishes may result from a pathogen attack or from unfavourable environmental factors. When their causes are known and the Koch's postulates have been fulfilled, these blemishes are called typical blemishes. The typical blemishes of pathogenic origin are due to various diseases caused by fungi, bacteria, nematodes or viruses. Black dot caused by *Colletotrichum coccodes*, silver scurf (*Helminthosporium solani*), skin spot (*Polyscytalum pustulans*), black scurf (*Rhizoctonia solani*), and powdery scab (*Spongospora subterranea*) are well known fungal diseases (Radtke and Rieckmann 1991; Stevenson et al. 2001). The most widely spread bacterial disease of potato in the world is due to *Streptomyces* spp. causing common scab and netted scab and the most frequently observed symptom due to nematode is stubby-root nematode lesions caused by *Paratrichodorus* spp. and *Trichodorus* spp. nematodes. Potato Virus Y^{ntn}, Tobacco Rattle Virus (TRV), and Tobacco Necrosis Virus (TNV) are also known to cause superficial blemishes on potato tubers. Abiotic factors such as humidity, temperature, light, chemical products, nutrient deficiency or mechanical damage cause enlarged lenticels, skin discoloration, tuber cracks or bruising. By contrast, the blemishes for which the causal agent has not been clearly identified are called atypical blemishes.

In a previous study (Fiers 2010), most of tuber blemishes were classified according to the type of symptom: sclerotia, enlarged lenticels, skinning, russeting, common scab, netted scab and atypical corky blemishes. The latter including corky cracks, corky spots or "rhizoscab", star-like corky lesions and blemishes commonly called "elephant hide", described as irregular polygonal sunken corky lesions (Fig. 1). These will hereafter be called polygonal lesions. Atypical blemishes frequently observed in ware potato production are the atypical corky blemishes, especially polygonal lesions and corky spots. Black scurf or sclerotia are known to be the long term survival form of *R. solani* (Anderson 1982; El Bakali and Martin 2006) and common and netted scab have been unequivocally demonstrated to be caused by *Streptomyces* spp. (Lambert and Loria 1989). These typical blemishes (sclerotia, common scab and netted scab) were integrated in this study as reference blemishes.

Production of all types of potato commodity aims at providing high quality tubers, either ware potatoes or seed tubers that need to meet the market demands related to the visual quality of the tubers. Atypical blemishes are then a predominant obstacle to the fulfilment of this quality requirement. Thus the determination of the causes of blemishes is needed. Assuming that atypical blemishes are of biological origin, two related hypotheses were considered: atypical blemishes are due to pathogenic microorganisms not yet identified, or they are due to known pathogens producing atypical symptoms. Some atypical blemishes closely resemble netted scab caused by *Streptomyces* spp. but they are also occasionally attributed to *R. solani* Kühn (teleomorph: *Thanatephorus cucumeris* (Franck) Donk) (Campion et al. 2003). This is the reason why *R. solani* and *Streptomyces* were investigated as well. The objectives of this study were: (1) to isolate potential pathogens from atypical skin blemishes, and (2) to test whether the isolated microorganisms are able to re-create the atypical blemishes on progeny tubers and so doing, verifying Koch's postulates.

Materials and methods

Plant material

Potato tubers were collected in 2006 and 2007 in 12 different French departments representing production bases for seeds as well as for ware potatoes. In 2006 and 2007, samplings were made in 51 and 39 fields, respectively. From each field, 1 to 4 tubers representative of the overall diversity of blemishes were chosen

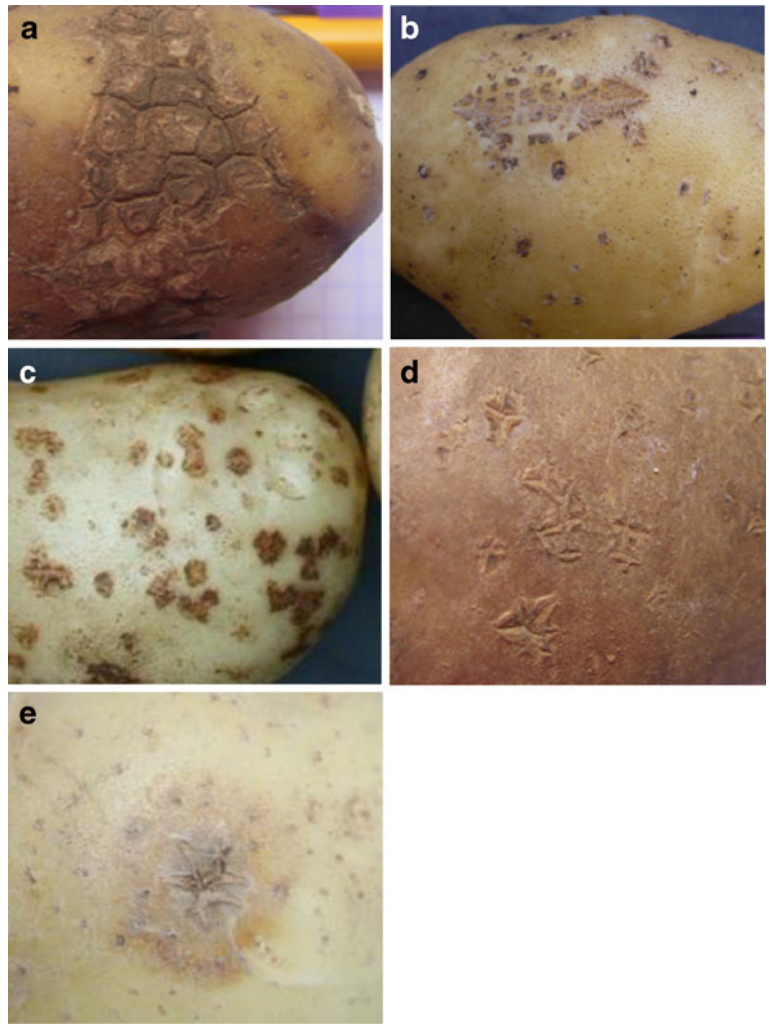

Fig. 1 Pictures of atypical corky blemishes. **a** Irregular polygonal sunken corky blemishes or polygonal lesions; **b** Corky crack; **c** Corky spot; **d** Star-like corky lesions without halo; **e** Star-like corky lesions with halo

for the study, resulting in a collection of 148 and 56 tubers sampled in 2006 and 2007, respectively. Though 42 different cultivars of potato were represented, the genetic background of *S. tuberosum* has been set aside deliberately in this study because the relationship between potato cultivar and soil-borne parasites are highly complex and still not fully understood. Blemishes were observed and classified into ten groups (Table 1). Atypical corky blemishes are illustrated in Fig. 1. The tubers were stored in paper bags at 4°C during several weeks until the start of the experiment.

Isolation of fungi

Tubers were washed under running tap water and air dried. A photograph of each affected tuber was taken. A 6 mm diameter and 5 mm deep piece was excised with a cork borer from the affected area of each tuber. The explants were surface sterilized in 1% bleach for 15 s and rinsed three times in sterile water. Each tuber explant was dried on sterile paper, and plated on potato dextrose agar (PDA). For the tubers collected in 2006, a second explant per tuber was taken and plated on water agar.

After 5 days of incubation at room temperature under natural light, fungal colonies developing from the plant material were identified by microscopic observations and purified at least twice by serial transfers on PDA. A total of 349 fungal isolates was recovered (Table 1) and stored both on PDA at room temperature and by cryopreservation at −80°C in the collection "Microorganisms of Interest for Agriculture and Environment" (MIAE, INRA Dijon, France).

Table 1 Fungal and *Streptomyces* species isolated from the different blemishes

Fungal or *Streptomyces* species	Sclerotia	Polygonal lesions	Corky cracks	Corky spots	Star-like corky lesions	Enlarged lenticels	Skinning	Russeting	Common scab	Netted scab	Other	Total
Absidia glauca							1					1
Alternaria arborescens		1										1
A. citri					1							1
A. longissima		1		1						1		3
Alternaria sp.	3	17		4	4	4	3	1	3		2	41
Bjerkandera adusta		1				1	1					3
Bjerkandera sp.		3					1					4
Ceratobasidium sp.				1								1
Cercophora grandiuscula						1						1
Cladosporium cladosporioides					1		1					2
Cladosporium sp.	1	1			1	1			1			5
Clonostachys rosea	2	12			1	6	1		5			27
Colletotrichum coccodes	1	2	1			1			2			7
Colletotrichum sp.		2										2
Cylindrocarpon olidum		1										1
Epicoccum nigrum					1				4	1	1	7
Fusarium avenaceum					1					1		2
F. culmorum	1											1
F. equiseti				1	1				1			3
F. graminearum	1						1		1			3
F. oxysporum	4	20	3	3	4	2	2		6	1	1	46
F. redolens		2				1						3
F. sambucinum						1	1				1	3
F. sambucinum or *F. tumidum*											1	1
F. solani			2			2					1	5
F. venenatum			1		2		1	1				4
Fusarium sp.		3		2		1			1	1	1	9
Gliomastix murmorum		1										1
Microdochium bolleyi			1	1					1			3
Microdochium sp.									1			1
Mortiella elongata										1		1
Mucor circenelloides	1	4				2			1		1	9
M. fragilis	1											1
M. hiemalis	1	1		1			1					4
Mucor sp.					1							1
Neonectria radicicola		1				1					2	4
Neonectria sp.		1										1
Penicillium brasilianum	1		1			1						3
P. brevicompactum	1	3		1		12		1	2	1		21
P. freii					1							1

Table 1 (continued)

Fungal or *Streptomyces* species	Sclerotia	Polygonal lesions	Corky cracks	Corky spots	Star-like corky lesions	Enlarged lenticels	Skinning	Russeting	Common scab	Netted scab	Other	Total
P. paneum										1		1
P. raistrickii						1						1
P. swiecickii		1										1
Penicillium sp.	1			1		2			1			5
Phoma exigua		1	2	1	2	1			1	2		10
Plectosphaerella cucumerina	1	4		2		5			1			13
Rhizoctonia solani AG 2-1	5								1			6
R. solani AG 3 - PT	29	9	1			6	2		7	1	1	56
R. solani AG 5				1		2		1				4
Rhizoctonia sp.				1	1							2
Rhizopus oryzae				1								1
Rhizopus sp.				1								1
Stereum rugosum							1					1
Trichocladium asperum										1		1
Trichoderma tomentosum										1		1
T. velutinum		1										1
T. viride		1										1
Trichoderma sp.							1	1				2
Ulocladium capsicum		1										1
Ulocladium sp.		1										1
Verticillium dahliae		1										1
Total fungi												349
Streptomyces scabiei									4			4
Streptomyces sp.		11							5	1		17
Total *Streptomyces*												21

Isolation of *Streptomyces*

Streptomyces spp. were isolated from tubers collected in 2007 showing skinning (1 tuber), common scab (5 tubers), star-like corky lesions (1 tuber), polygonal lesions (16 tubers) and netted scab (4 tubers). Isolations were made according to the method described by Bouchek-Mechiche et al. (2000). Tubers were washed under tap water, disinfected in ethanol from 1 min for very superficial blemishes to 5 min for deeper blemishes. They were rinsed in two consecutive baths of sterile water and air dried for at least 3 h. About 50 mg of affected skin was excised by scraping the tuber surface with a sterile scalpel and collected in a sterile mortar. One hundred µl of sterile water was aseptically added, and the mixture was homogenised with a pestle; then, about 400 µl of sterile water was added to get a smooth and homogenous mixture. After serial dilutions in sterile water, 200 µl of dilutions 10^{-3} and 10^{-5} for superficial blemishes and dilutions 10^{-4} and 10^{-6} for deep blemishes were deposited in a 9 cm Petri dish. Twenty ml of tyrosine, sodium caseinate, sodium nitrate (TCN) medium (1 gl^{-1} of L-tyrosine, 25 gl^{-1} of sodium caseinate, 10 gl^{-1} of sodium nitrate, 15 gl^{-1} of agar) maintained at 45°C were added. Four replicates per dilution were made. Plates were incubated at 27°C for 10 days. Each colony of *Streptomyces* was transferred to PDA and stored at 4°C. A total of 21 isolates of *Streptomyces* were collected from 27 tubers collected in 2007 (Table 1).

Molecular identification of fungal isolates

For DNA extraction, all the collected fungal isolates were cultivated in tubes on PDA slants. Two ml of potato dextrose broth (PDB) were poured into PDA tubes and vortexed to disperse the spores, and the spores-PDB mix was poured into Roux flasks containing 100 ml of PDB. For non-sporulating fungi, six explants of PDA were directly placed into Roux flasks. Flasks were incubated at room temperature without shaking for 2 to 3 days. The mycelium was harvested by filtration, frozen at −80°C during 30 min, lyophilized and stored at −80°C.

The mycelium was ground in liquid nitrogen in a sterile mortar to obtain a mycelium powder. The DNA was extracted from 20 mg of mycelium powder using DNeasy plant mini kit (Qiagen, Courtaboeuf, France) according to the manufacturer's protocol. The DNA quantity and quality were checked by electrophoresis on a 0.8% agarose gel, revealed with ethidium bromide and visualized by UV trans-illumination. The DNA concentrations calculated with the image analysis software Bio-Profil Bio1D++ (Windows Application V11.9, Copyright ©2004 Vilbert-Lourmat) were between 3.5 and 125 ng/µl.

For each fungal isolate, the internal transcribed spacer (ITS) region of the ribosomal DNA (rDNA) was amplified by PCR with the primers ITS1-F (CTTGGTCATTTAGAGGAAGTAA) and ITS4 (TCCTCCGCTTATTGATATGC) (Gardes and Bruns 1993; White et al. 1990). PCR amplifications were performed in a final volume of 50 µl by mixing 2 µl of DNA with 0.5 µM of each primer, 150 µM of dNTP, 6 U of *Taq* DNA polymerase (Q-Biogen, Evry, France) and PCR reaction buffer. Amplification was conducted in a mastercycler (Eppendorf, Hambourg, Germany) with an initial denaturation of 3 min at 94°C, followed by 35 cycles of 1 min at 94°C, 1 min at 50°C, 1 min at 72°C, and a final extension of 10 min at 72°C. Aliquots of PCR products were checked by electrophoresis on a 1% agarose gel, revealed with ethidium bromide and visualized by UV trans-illumination.

The PCR products were sequenced by Beckman Coulters Genomics (Takeley, UK) using primers ITS1-F and ITS4. For each PCR product, sequences from both strands were assembled to produce a consensus sequence. Sequence identities were determined using BLAST analyses from the National Center for Biotechnology Information (NCBI) available on line.

Molecular identification of *Streptomyces* isolates

Streptomyces isolates stored at 4°C on PDA were cultivated in 25 ml of Luria Bertani (LB) media (10 $g l^{-1}$ of bacto tryptone, 5 $g l^{-1}$ of yeast extract, 10 $g l^{-1}$ of NaCl; pH 7) for 6 days at 27°C. The DNA was extracted using the DNeasy Blood and Tissue kit (Qiagen) according to the manufacturer's specifications. The DNA quantity and quality were checked by electrophoresis as above and the DNA concentrations calculated as above were between 4 and 650 ng/µl.

For each *Streptomyces* isolate, the 16S rDNA was amplified by PCR with the primers 27F (AGAGTTT GATCCTGGCTCAG) (Edwards et al. 1989) and 1392R (ACGGGCGGTGTGTACA) (Braker et al. 2001). PCR reactions were performed in a final volume of 50 µl by mixing 10 µl of DNA with 0.2 µM of primer 27F, 0.2 µM of 1392R, 200 µM of dNTP, 12 U of *Taq* DNA polymerase (Q-Biogen) and PCR reaction buffer. Amplifications were conducted in a mastercycler (Eppendorf) with an initial denaturation of 3 min at 95°C, followed by 35 cycles of 1 min at 95°C, 1 min at 57°C, 1 min at 72°C, and a final extension of 5 min at 72°C. Aliquots of PCR products were checked by electrophoresis on a 1% agarose gel as above.

The PCR products were sequenced using primers 27F and 1392R. For each PCR product, sequences from both strands were assembled to produce a consensus sequence. Sequences identities were determined using BLAST as above.

Pathogenicity tests

Isolates representative of the most frequently isolated fungal and *Streptomyces* species were tested for their pathogenicity on potato tubers. In order to fulfil Koch's postulates for these isolates, two different types of bioassays were set up: a pot-test with artificially infested soil and a test where potatoes were grown under hydroponic conditions.

Bioassays in soil

The bioassays were conducted in 2007 and 2008 in a greenhouse from April to September. Healthy potato

tubers were grown in pots containing soil artificially infested with fungal or *Streptomyces* isolates. Thirty-three isolates were tested in 2007 and 48 in 2008. Nine isolates were tested twice (Table 2). In addition, four reference isolates were tested: *Fusarium sambucinum* (TFSa, isolated in France 2007), *F. solani* var. *coeruleum* (T FSC1, isolated in France 2006) *R. solani* AG 3 (i4/9729-1, isolated in France 1999), and *R. solani* AG 2-1 (0799-001 2 N WA, isolated in Morocco 2007).

Fungal inoculum was prepared on autoclaved millet seeds. Forty grams of seeds were mixed in jars with 32 ml of sterile deionised water and autoclaved for 45 min at 110°C on two consecutive days and for 20 min at 120°C the third day. The jars were stored at room temperature during 4 days before inoculation to allow the release of putative toxic compounds (mainly NH_3). Six plugs of 12 day-old fungal cultures on PDA were introduced and mixed with the millet seeds. The cultures were incubated at room temperature for 3 weeks with regular shaking.

For the *Streptomyces* inoculum, *Streptomyces* spp. were grown on 9 cm PDA plates. Prior to the inoculum setting, a specific substrate for the *Streptomyces* inoculum was prepared. One litre of vermiculite, mixed with 150 ml of deionized water was autoclaved for 1 h at 120°C, on two consecutive days. 170 ml Say's media (sucrose 20 gl^{-1}; asparagine 1.2 gl^{-1}; K_2HPO_4 0.6 gl^{-1}; yeast extract 10 gl^{-1}) was added to the vermiculite and autoclaved for 30 min at 115°C. *Streptomyces* mat and spores were scraped from 7 to 10 day-old culture on oatmeal agar (oatmeal powder 50 gl^{-1}; agar 23.5 gl^{-1}) at 27°C and ground in a sterile mortar with 8 ml sterile water. The resulting mixture was poured into the sterile vermiculite substratum and was incubated for 2 to 3 weeks at 27°C with daily shaking (Wanner 2004).

The potting mixture (one third of sand and two thirds of peat) used to grow the potatoes was steam-disinfected and stored at room temperature for 7 days to allow putative toxic compounds to be released. The fungal and *Streptomyces* inocula were mixed separately with about 6 l of disinfected potting mixture with a three-dimensional shaker (Turbula, System Schatz). The infested potting mixture was placed in 10 l plastic pots (25 cm diameter, 30 cm high). The pots infested with *Streptomyces* were prepared 2 weeks before planting, covered with a plastic cover and kept at room temperature for about 15 days to allow the establishment and multiplication of the bacteria.

One seed tuber was planted in each pot. Cultivar Charlotte was chosen for soil assays because it was one of the cultivars most cited in the sample collections (2006 and 2007) and because it has a medium to high overall susceptibility to atypical superficial blemishes (FNPPPT and GNIS 2010, Chatot, personnal data). Commercial certified seeds (Class A; 25–32) were used and visually examined for absence of any skin blemish. Plants were grown in a glasshouse at room temperature with minimal temperature of 10°C and maximal temperature of 25°C with a 16 h day length, for approximately 4 months, until natural maturity; additional light (200 W/m^2) was provided when needed. Plants were regularly watered, but soil moisture content was not monitored. Fertilizing watering was carried out every week with a fertilizer solution (N P K, 20 20 20). Plants were harvested in September. A non inoculated control and three replicate pots per treatment were set up.

At harvest, 1 g of soil from each pot was spread on PDA in a Petri dish to check the survival and viability of the inoculated fungi and bacteria during the assay. After 4 days, the presence of the inoculated micro-organisms was checked under a microscope.

Progeny tubers from the same plant were washed under running tap-water, air-dried, weighed and stored in a paper bag. The number of tubers per plant was recorded and the tubers larger than 3 cm long were scored individually according to the different classes of blemishes. The scoring scale edited by the French official service of control and certification (SOC) (GNIS and SOC 2009) was adapted to black scurf, netted scab and common scab scales, each with 10 different levels of disease severity. Black scurf scale was: 0 = no lesion, 1=1% of area covered by lesions; 3=4% of area covered by lesions; 5=9% of area covered by lesions; 7=14% of area covered by lesions; 9=35% of area covered by lesions. Netted and common scab scales scored tuber as : 0 = no lesion, 1=4% of area covered by lesions; 3=6% of area covered by lesions; 5=30% of area covered by lesions; 7=45% of area covered by lesions; 9=60% of area covered by lesions

The intensity of sclerotia was scored according to the *R. solani* scale, from level 1 (1% of the tuber surface covered by the blemish) to level 9 (>35% of

Table 2 Strains tested in the two bioassays conducted in infested soil

Species	Strains[a]	MIAE accession number[b]	Host of origin (potato cultivar)	Blemish of origin	Bioassays results		
					Observed blemishes		Detection of the inoculated species on the tubers[d]
					Identical to the original one (intensity[c])	Different from the original one	
Alternaria sp.	0629-002 J 1B PDA	MIAE00160	Juliette	Star-like corky lesion		Skinning, enlarged lenticels	−
	0629-002 M 2A PDA	MIAE00162	Marine	Polygonal lesions		Skinning, enlarged lenticels	+
	0629-041 1B PDA	MIAE00096	Daisy	Corky spot		Skinning, enlarged lenticels	−
	0629-045 3B WA	MIAE00099	Isabelle	Polygonal lesions	Polygonal lesions (1)	Skinning, enlarged lenticels	−
	0629-058 2 PDA	MIAE00182	Samba	Polygonal lesions	Polygonal lesions (1)		−
	0629-058 3A PDA *	MIAE00183	Samba	Polygonal lesions	Polygonal lesions (1)		−
	0629-059 1Aβ WA	MIAE00184	Fuego	Skinning	Skinning (2)	Enlarged lenticels	−
Cladosporium sp.	0629-022 2 PDA	MIAE00169	Chérie	Common scab		Polygonal lesions	−
Clonostachys rosea	0628-013 3 PDA	MIAE00156	Anoe	Enlarged lenticels	Enlarged lenticels (1)	Skinning, star-like corky lesions	−
	0629-022 1 WA	MIAE00210	Charlotte	Common scab		Skinning, enlarged lenticels	−
	0629-023 3A WA	MIAE00199	Charlotte	Common scab		Skinning, enlarged lenticels, polygonal lesions	−
	0629-030 1 WA	MIAE00175	Samba	Polygonal lesions		Skinning, enlarged lenticels	−
	0629-038 2 WA	MIAE00084	Samba	Polygonal lesions		Skinning, enlarged lenticels	−
	0629-038 4 PDA *	MIAE00088	Samba	Polygonal lesions	Polygonal lesions (1)		−
	0629-040 3C WA	MIAE00095	Samba	Polygonal lesions		Skinning, enlarged lenticels	−
	0629-056 1 PDA	MIAE00180	Spunta	Enlarged lenticels	Enlarged lenticels (1)	Skinning, sclerotia	−
Colletotrichum coccodes	0610-001 2A PDA	MIAE00075	Daifla	Corky crack		Skinning, polygonal lesions	+
Fusarium equiseti	0629-023 1B PDA	MIAE00079	Charlotte	Common scab		Polygonal lesions	+

F. oxysporum	0628-015 1A PDA	MIAE00157	Amandine	Corky spot		Skinning, enlarged lenticels	−
	0629-002J 2Bβ PDA *	MIAE00163	Juliette	Star-like corky lesions		Skinning, enlarged lenticels	+
	0629-023 3B PDA	MIAE00172	Charlotte	Common scab		Skinning, enlarged lenticels	−
	0629-024 2 PDA	MIAE00173	Désirée	Common scab		Skinning, enlarged lenticels	−
	0629-040 1A PDA	MIAE00091	Samba	Polygonal lesions		Skinning, enlarged lenticels	−
	0629-040 2B WA	MIAE00214	Samba	Polygonal lesions	Polygonal lesions (1)	Skinning, enlarged lenticels	+
	0629-055 1 WA	MIAE00178	Pamela	Corky spot		Skinning, enlarged lenticels	−
	0629-058 1 PDA	MIAE00181	Samba	Star-like corky lesions		Skinning, enlarged lenticels	−
	0629-067 2 WA	MIAE00186	Samba	Polygonal lesions		Skinning, enlarged lenticels	+
F. sambucinum/*F. tumidum*	0628-012 3 PDA	MIAE00209	Charlotte	Dry rot		Skinning	+
F. sambucinum	T FSa **		unknown	Dry rot		Skinning, star-like corky lesions	+
F. solani var. *coeruleum*	T FSC 1 **		unknown	Dry rot		Polygonal lesion	−
F. solani	0610-001 2 WA	MIAE00074	Daifla	Corky crack		Skinning, enlarged lenticels, sclerotia	+
	0610-004 2 WA	MIAE00201	Nicola	Corky crack		Skinning, enlarged lenticels	+
F. venenatum	0610-001 1A PDA	MIAE00070	Daifla	Star-like corky lesions		Skinning, enlarged lenticels	−
	0610-004 1 PDA *	MIAE00076	Nicola	Corky crack		Skinning, polygonal lesions	−
Fusarium sp.	0629-021 2A PDA	MIAE00167	Atlas	Mould lesion		Skinning	+
Microdochium sp.	0629-024 2 WA	MIAE00174	Désirée	Common scab		Polygonal lesions, skinning, enlarged lenticels	−
Neonectria radicicola	0628-019 1A WA	MIAE00158	Anoe	Enlarged lenticels		Polygonal lesions	−
Penicillium brevicompactum	0628-006 3B PDA	MIAE00151	Amandine	Enlarged lenticels	Enlarged lenticels (1)	Skinning, polygonal lesions	−
	0628-012 1A WA	MIAE00153	Charlotte	Enlarged lenticels	Enlarged lenticels (1)	Skinning	+
	0628-013 2	MIAE00215	Anoe	Enlarged	Enlarged lenticels (1)	Skinning, polygonal lesions	−

Table 2 (continued)

Species	Strains[a]	MIAE accession number[b]	Host of origin (potato cultivar)	Blemish of origin	Bioassays results		
					Observed blemishes		Detection of the inoculated species on the tubers[d]
					Identical to the original one (intensity[c])	Different from the original one	
	WA			lenticels			
Penicillium sp.	0628-001 1B PDA	MIAE00149	Adriana	Enlarged lenticels		Skinning, polygonal lesions	+
Plectosphaerella cucumerina	0629-039 1 WA	MIAE00089	Amandine	Enlarged lenticels	Enlarged lenticels (2)	Polygonal lesions	+
Rhizoctonia solani AG 2.1	0799-001 2N WA **	MIAE00189	Nicola	Corky crack		Skinning, enlarged lenticels	−
R. solani AG 3 - PT	0602-001 1B PDA *	MIAE00072	Mixed	Polygonal lesions	Polygonal lesions (1)	Sclerotia, skinning	−
	0628-006 1A WA	MIAE00150	Amandine	Enlarged lenticels	Enlarged lenticels (2)	Skinning, polygonal lesions, sclerotia	+
	0628-006 3A PDA	MIAE00219	Amandine	Enlarged lenticels		Skinning, polygonal lesions, sclerotia	+
	0629-004 1A WA *	MIAE00164	Spunta	Sclerotia	Sclerotia (3)	Polygonal lesions	+
	0629-014 3 WA	MIAE00165	Chérie	Common scab		Skinning, enlarged lenticels, polygonal lesions, sclerotia	+
	0629-017 1 PDA	MIAE00166	Charlotte	Polygonal lesions	Polygonal lesions (2)	Sclerotia	+
	0629-023 1A PDA	MIAE00170	Charlotte	Common scab		Polygonal lesions, sclerotia	+
	0629-030 2 PDA	MIAE00176	Samba	Polygonal lesions	Polygonal lesions (1)	Sclerotia	+
	0629-030 3 PDA	MIAE00081	Samba	Polygonal lesions	Polygonal lesions (1)	Sclerotia	+
	0629-033 2B WA *	MIAE00082	Juliette	Skinning		Enlarged lenticels, sclerotia	+
	0629-036 1A WA	MIAE00083	Juliette	Skinning	Skinning (6)	Polygonal lesion, sclerotia	−
	0629-038 3A WA *	MIAE00087	Samba	Sclerotia	Sclerotia (2)	Skinning, polygonal lesions	+
	0629-039 2A WA	MIAE00090	Amandine	Enlarged lenticels	Enlarged lenticels (2)	Skinning, polygonal lesion, sclerotia	+
	0629-040 2A WA *	MIAE00092	Samba	Polygonal lesions	Polygonal lesions (1)	Sclerotia	+
	0629-049 2	MIAE00006	Juliette	Polygonal		Skinning, enlarged lenticels,	+

	WA			lesions		sclerotia	
	0629-055 3 WA *	MIAE00179	Pamela	Sclerotia	Sclerotia (2)	Polygonal lesions	+
	0629-059 3 PDA	MIAE00185	Fuego	Corky crack	Corky crack (2)	Skinning, sclerotia	+
	i 4 */**		unknown	Sclerotia	Sclerotia (2)	Skinning, polygonal lesions	+
R. solani AG 5	0628-023 1B WA	MIAE00213	Chérie	Enlarged lenticels	Enlarged lenticels (1)	Skinning, polygonal lesions, sclerotia	−
Streptomyces scabiei	B M_ 0745-001 P		Bintje	Common scab		Skinning, enlarged lenticels	−
Streptomyces sp.	E M1_ 0747-002 P		Yona	Common scab		Skinning, enlarged lenticels	+
	F M1_ 0729-049		Rosabelle	Netted scab		Skinning, enlarged lenticels	−
	H P _0762-005		Bintje	Netted scab		Skinning, enlarged lenticels	−
	L M_ 0756-093		May Flower	Netted scab		Skinning, enlarged lenticels, common scab	−
	L P_ 0756-093		May Flower	Netted scab		Skinning, enlarged lenticels	−
	M P1_ 0729-019		Urgenta	Common scab	Common scab (1)	Skinning, enlarged lenticels	−
	V P2 _0762-007		Bintje	Netted scab		Skinning, enlarged lenticels	−
Non-inoculated control						Skinning, enlarged lenticels, polygonal lesions	

[a] The codification of the strains includes the year of isolation (two first digits), the geographical zone of isolation corresponding to the French departments (third and fourth digits) and the strain identification number (last digits and letters). Strains followed by an asterisk (*) were tested in the two different bioassays (2007; 2008). Strains followed by two asterisks (**) are reference strains, which were not sequenced in this study

[b] Collection MIAE, Microorganisms of Interest for Agriculture and Environment (INRA Dijon, France)

[c] Intensities of blemishes were scored according to the official French scales of the tuber potato diseases (GNIS and FNPPPT). Sclerotia were scored from level 1 (1% of the tuber surface covered by the blemish) to level 9 (>35% of the tuber surface covered by the blemish) and the other blemishes were scored from level 1 (4% of the tuber surface covered by the blemish) to level 9 (>60% of the tuber surface is covered by the blemish)

[d] + means that the inoculated strain was isolated from the progeny tubers; − means that the inoculated strain was not isolated from the progeny tubers

the tuber surface covered by the blemish). Other blemishes were scored according to the common and netted scab scale, from level 1 (4% of the tuber surface covered by the blemish) to level 9 (>60% of the tuber surface covered by the blemish). The intensity was calculated by averaging the intensity of the reproduced blemish for the three replicates (six when the isolate was tested twice) (Table 2). Fungi and *Streptomyces* were isolated from the progeny tubers following the same protocol as above.

Co-inoculation tests under hydroponic conditions

In the second type of test, potatoes were grown under hydroponic conditions in order to follow the development of the blemishes *in-situ*. A total of 22 isolates of fungi and *Streptomyces* spp. were tested in single and co-inoculations, resulting in 49 different treatments (Table 3).

The experimental system (adapted from Gray 1973) (Fig. 2) consisted of a tray (40 cm in diameter and 10 cm in depth) with a 5 cm diameter hole in the centre, placed on a 5 l pot containing nutrient solution (N P K, 13 21 13 or N P K, 8 17 24, see below) (Algospeed Flo, Compo, France). A seed tuber was placed in the centre of the tray on a plastic screen of 1 cm mesh, maintaining the tuber in the tray, away from the water but allowing the roots to elongate into the nutrient solution. Certified seeds (Class A, 28–35) of the cultivar Bintje were used; this cultivar is known for its susceptibility to overall skin blemishes

A sterile mixture of 7 l of perlite and vermiculite (50/50 v/v) was placed in the tray as to completely cover the seed tuber; it was regularly moistened to initiate the development of roots and stolons. Stems were supported by a stick. All pots were set in a greenhouse; the day length was 12 h 30 min and temperature was kept at 15°C during the day and 10°C during the night. After 2 weeks, when sprouting was initiated the perlite-vermiculite mixture was removed and the tray was covered with a black foam disc to keep the newly formed tubers in the dark. During the course of the experiment, nutrient solution was aerated with an air pump (Hiblow, Airpump Takatsuki). The nutrient solution (N P K, 13 21 13) was used during the initiation of the stolons with a high content in phosphorus to favour root development. After 2 months it was changed to (N P K, 8 17 24), a solution with more potassium to strengthen tubers. The pots were emptied and filled again with fresh nutrient solution (pH≈6 and conductivity≈ 1500 μS) once a week.

Two and a half months after plantation, when progeny tubers reached a sufficient size (i.e. >2 cm long), they were inoculated with one or two isolates. Inoculations were performed using plugs of 10 day-old microbial cultures on PDA plates. Six visually healthy progeny tubers per plant were chosen. Three of them were wounded with a sterile toothpick and the three others were not. For each treatment, plugs of the isolates were placed side by side on the tubers, on the wound for wounded tubers. For each treatment corresponding to single or double inoculations, three independent plants were inoculated. A control treatment corresponding to three non-inoculated plants was also included. Plants were grown until natural senescence and harvested individually.

After inoculation, newly formed tubers were observed weekly during 7 weeks. The blemishes were scored when they appeared and throughout their development.

At harvest, tubers from each plant were washed under running tap-water, air-dried and placed in a paper bag. The number of tubers per plant was recorded and the tubers were scored individually for atypical corky blemishes with the scoring scale from 1 to 9.

Fungal and *Streptomyces* isolations on progeny tubers were done following the same protocol as above.

Results

Among the samples collected in 2006 and 2007, the blemishes most frequently observed were polygonal lesions, representing 22% of the total blemishes, followed by sclerotia (16%), enlarged lenticels (15%), corky spots (13%), common scab (8%), corky cracks (8%), star-like corky lesions (7%), skinning (5%), netted scab (4%), and russeting (2%). The atypical corky blemishes (polygonal lesions, corky spots, corky cracks and star-like corky lesions) together represented 50% of all observed blemishes.

Identification of fungi

Twenty six different fungal genera were found among the 349 isolates collected from blemishes and identified from their ITS sequence (Table 1). The most

Table 3 Strains tested in the bioassay conducted with single and co-inoculations under hydroponic conditions

Species	Strains	MIAE accession number	Host of origin (potato cultivar)	Blemish of origin	Observed blemishes in the bioassay[a]							
					Single inoculations	Double inoculations[b]						
						R. solani AG 3 0629-023 1A PDA	*Fusarium oxysporum* 0629-023 3B PDA	*Alternaria* spp. 0629-048 1B PDA	*Penicillium brevicompactum* 0629-056 3A PDA	*Streptomyces* spp.		*Plectosphaerella cucumerina* 0629-023 1A WA
										A M1_0729-008	L P_0756-093	
Alternaria spp.	0629-045 2B PDA	MIAE00191	Isabelle	Polygonal lesions	C, I, SL	NT	NT	NT	NT	NT	NT	NT
	0629-048 1B PDA	MIAE00192	Spunta	Skinning	C, G, I, L, P, S, SL	C, G, I, P, S	C, I, L, P, SL	NT	NT	NT	NT	NT
	0629-056 2B PDA	MIAE00193	Spunta	Enlarged lenticels	L, P	NT	NT	NT	NT	NT	NT	NT
	0629-058 3B WA	MIAE00194	Samba	Polygonal lesions	L, S, SL	NT	NT	NT	NT	NT	NT	NT
Bjerkandera adusta	0629-048 1Bβ WA	MIAE00195	Spunta	Skinning	I, L, P, S	NT	NT	NT	NT	NT	NT	NT
Clonostachys rosea	0629-023 3A WA	MIAE00199	Charlotte	Common scab	C, I, L, P, S, SL	NT	NT	NT	NT	NT	NT	NT
	0629-048 1Bα WA	MIAE00196	Spunta	Skinning	C, I, P	G, I, L, P, S	C, L	I, L, P, S	C, G, I, L, P, S, SL	C, I, L, P, SL	C, I, L, S, SL	C, I, L
Fusarium equiseti	0629-023 1B PDA	MIAE00079	Charlotte	Common scab	C, I, L, P, rot	NT	NT	NT	NT	NT	NT	NT
F. oxysporum	0629-023 3B PDA	MIAE00172	Charlotte	Common scab	C, I, G, L, P, S	I, L, S	NT	NT	NT	NT	NT	NT
	0629-067 2A PDA	MIAE00197	Samba	Polygonal lesion	I, L, P	NT	NT	NT	NT	NT	NT	NT
F. solani	0628-006 1A PDA	MIAE00198	Amandine	Enlarged lenticels	C, L, P, rot, S	NT	NT	NT	NT	NT	NT	NT
Penicillium brevicompactum	0628-006 2A WA	MIAE00003	Amandine	Enlarged lenticels	C, I, L, P, S	NT	NT	NT	NT	NT	NT	NT
	0629-056 3A PDA	MIAE00200	Spunta	Enlarged lenticels	C, I, P, S	C, I, L, P, S	C, L, P, S, SL	I, L, P	NT	NT	NT	NT
	0729-008 PDA	MIAE00004	Naga	Polygonal lesion	C, I, P	NT	NT	NT	NT	NT	NT	NT
Plectosphaerella cucumerina	0629-023 1A WA	MIAE00202	Charlotte	Common scab	C, I, L, P, SL	C, I, L, P, S	I, L, P	C, G, I, L, P, S, SL	C, I	C, I, L, P, S	I, L	NT
	0680-006 2Bα WA	MIAE00203	Hybride A	Sclerotia	C, G, L, P, S, SL	NT	NT	NT	NT	NT	NT	NT
Rhizoctonia solani AG 2-1	0680-006 2Aβ WA	MIAE00208	Hybride A	Sclerotia	G, I, L, SL	NT	NT	NT	NT	NT	NT	NT
R. solani AG 3 - PT	0629-023 1A PDA	MIAE00170	Charlotte	Common scab	C, I, L, P, S	NT	NT	NT	NT	NT	NT	NT
Streptomyces scabiei	Y P1_0747-001 P	MIAE00204	Hybride B	Common scab	C, I, P, S	NT	NT	NT	NT	NT	NT	NT
Streptomyces spp.	A M1_0729-008	MIAE00205	Naga	Polygonal lesion	G, L, rot, S	C, I, L, P, S	C, I, L, P, S	L, P, S	C, I, L, P, rot, S, SL	NT	NT	NT

Table 3 (continued)

Species	Strains	MIAE accession number	Host of origin (potato cultivar)	Blemish of origin	Observed blemishes in the bioassay[a]							
					Single inoculations	Double inoculations[b]						
						R. solani AG 3 0629-023 1A PDA	*Fusarium oxysporum* 0629-023 3B PDA	*Alternaria* spp. 0629-048 1B PDA	*Penicillium brevicompactum* 0629-056 3A PDA	*Streptomyces* spp.		*Plectosphaerella cucumerina* 0629-023 1A WA
										A M1_0729-008	L P_0756-093	
	L P_0756-093	MIAE00206	May Flower	Netted scab	C, G, I, L, P, S, SL	C, I, L, P, S, SL	C, G, P, S	G, I, L, P, S	C, G, I, L, P, S	NT	NT	NT
	X M_0747-001 L	MIAE00207	Hybride B	Netted scab	C, I, P, S	NT	NT	NT	NT	NT	NT	NT
Non-inoculated control				I, L								

[a] Observed blemishes are scored with the following abbreviations. *C* corky cracks, *G* greening, *I* Polygonal lesion, *L* enlarged lenticels, *P* Tuber becoming pink, *S* Skinning, *SL* Star-like corky lesion

[b] NT, not tested

represented genera were *Fusarium* (80 isolates), *Rhizoctonia* (68), *Alternaria* (46), *Penicillium* (33), and *Clonostachys* (27). Most of the isolates were identified at the species level, however, some isolates were only identified at the genus level, because either their ITS sequence had the same percentage of similarity as two or more fungal species or the obtained ITS sequence was too short. Finally, at least 45 different fungal species were identified. In the case of *R. solani* isolates, the anastomosis groups (AG) were also determined based on ITS sequences (Table 1).

Identification of *Streptomyces*

The 21 different *Streptomyces* isolates were collected from polygonal lesions (11), common scab (9), and netted scab (1). No *Streptomyces* were isolated from the tuber presenting skinning or from the tuber presenting star-like corky blemishes. Among the 21 isolates of *Streptomyces*, four were identified as *S. scabiei* (formerly *S. scabies*) from their 16S rDNA sequence with 99 or 100% of similarity with the reference strains (Table 1). The isolates of *S. scabiei* were especially associated with common scab. For the other isolates, the 16S rDNA sequence obtained was too short to allow the identification at the species level.

Pathogenicity tests

Bioassays in soil

The inoculated strains were re-isolated from the soil in 39% of the cases. Fungi belonging to the *Rhizoctonia solani* species were more frequently re-isolated than the other species.

The results of the two bioassays conducted in soil are presented in Table 2. Among the 62 fungal isolates and the 8 *Streptomyces* isolates inoculated, 30 fungi and 1 *Streptomyces* were re-isolated from the progeny tuber surfaces at the end of the tests. Several other fungi that were not initially inoculated in pots were also isolated such as *Trichoderma* spp., *Mucor* spp. and *Colletotrichum* spp. Among the inoculated fungi, *R. solani* was the species the most frequently re-isolated from tubers (16 out of 21 were recovered on the progeny tuber surface). Conversely, only one out of the 7 *Alternaria* isolates tested and none of the

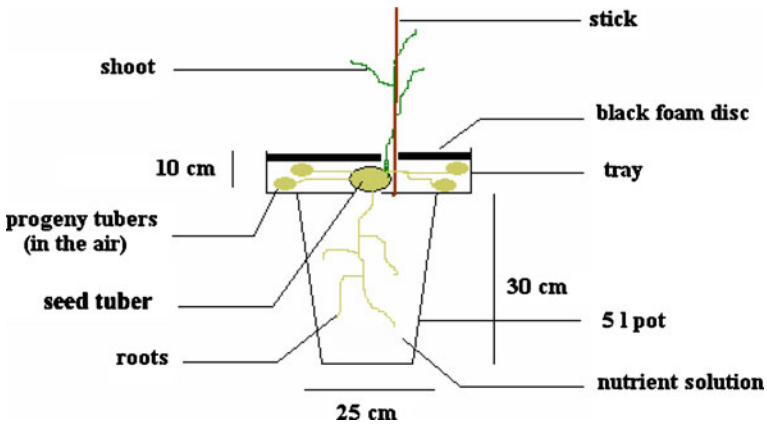

Fig. 2 Scheme of the experimental set up of the assay under hydroponic conditions

8 *Clonostachys* isolates tested were recovered from the progeny tuber surface.

In all the treatments, all types of blemishes were observed with a variable intensity, but the original blemishes were rarely reproduced by the inoculated isolates. There was no clear relationship between the type of blemish and the taxonomic status of the isolates. However the isolates of *R. solani* AG 3 were able to cause sclerotia (four of the isolates) as well as polygonal lesions (four other isolates) and one isolate generated corky crack, on the progeny tubers. One of nine *F. oxysporum* isolates was able to cause polygonal lesions. One of the three isolates of *P. brevicompactum,* two isolates of *R. solani*, and the only isolate of *P. cucumerina* caused enlarged lenticels. Among the isolates of *Streptomyces* tested, none has produced the same blemish as the one from which it was isolated. Progeny tubers coming from the non-inoculated controls also showed blemishes: skinning (6 out of 6 tubers), enlarged lenticels (5 out of 6 tubers), and polygonal lesions (1 out of 6 tubers).

The intensity of reproduced blemishes varied from 1 to 6 with an average of 1.6, which means that in average, less than 6% of tuber surfaces was covered by a given type of blemish. In general, blemishes intensities were low. Some tubers showed several different types of blemishes making it difficult to make a connection between the isolated strain and a specific blemish.

Co-inoculation tests under hydroponic conditions

Before inoculation, 43% of the progeny tubers already displayed polygonal lesions with an average intensity of three, meaning that 6% of the tuber surface was covered by the blemish. The blemishes observed during the 7 weeks following the inoculation, are listed in Table 3. Corky cracks, polygonal lesions, enlarged lenticels, skinning, and star-like corky blemishes were produced on progeny tubers grown under hydroponic conditions and inoculated with one or two selected strains. For each treatment, a variety of blemishes was observed without any relationship between the strain inoculated and blemishes. In none of the treatments, the inoculated fungus or *Streptomyces* were re-isolated from progeny tubers after harvest. However, numerous *Mucor* spp. and *Penicillium* spp. were isolated. The diversity of blemishes and of fungi isolated from the non-inoculated tubers was similar to the one from the inoculated tubers.

Growing tubers under hydroponic conditions allowed the observation of formation and development of blemishes during tuber growth. It often appeared, on inoculated tubers as well as on non-inoculated tubers that wound and opened lenticel healing formed polygonal and star-like corky lesions. We also observed that polygonal lesions and corky cracks frequently resulted from the development of skinning and star-like corky lesions along with tuber growing period. Finally, crack formations were observed at the end of the growing period, when tubers tended to grow faster.

Discussion

The objective of this study was to investigate the microbial origin of potato skin tuber blemishes. We made a large collection of tubers showing different types of blemish, and isolated and identified micro-

organisms associated with these blemishes. At least 45 different species of fungi and one species of *Streptomyces* were isolated. *Fusarium*, *Rhizoctonia*, *Alternaria*, *Penicillium* and *Clonostachys* were the genera most frequently isolated. *Fusarium*, *Rhizoctonia* and *Alternaria* are known to be present at the surface of potato tubers and in the rhizosphere of potato plants (Cwalina-Ambroziak 2002; Pieta and Patkowska 2003). They are known to be responsible for dry rots, black scurf and stem canker and early blight, respectively, on potato crop (Radtke and Rieckmann 1991; Stevenson et al. 2001). *Clonostachys*, especially *C. rosea* (formerly *Gliocladium roseum*, Schroers et al. 1999) could be either a pathogenic agent causing dry rot (Theron 1991) or a biological agent controlling several potato soil-borne diseases (Davide and Zorilla 1995; Keinath et al. 1991). *Penicillium* species were isolated from potato tubers and identified as saprophytic strains (Cwalina-Ambroziak 2002). To our knowledge it is the first time that *P. brevicompactum,* the most common *Penicillium* species found in our study, was isolated from potato tubers. Thus, tubers are an ecological niche favourable for the development of a diversity of fungi and bacteria, both pathogens and saprophytes.

Though quite diverse, the microflora present at the surface of tubers displaying blemishes might have been underestimated by the isolation technique. Extracting the DNA directly from the tuber blemishes could have revealed an even larger spectrum of microorganisms. However, such a direct approach was not adapted to our purpose since it would not have allowed us to isolate the microorganisms, characterize and test them individually in bioassays.

Only one sample of each blemish type, skinning and star-like corky lesion, was analysed for the isolation of *Streptomyces*. No *Streptomyces* were isolated from these blemishes. Further research needs to be done to know if *Streptomyces* does interfere with the formation of skinning and star-like corky lesions, or not.

A diversity of fungi was isolated in this study, direct observation using a microscope often allowed their identification at the genus level but further identification at the species level frequently required molecular tools. The ITS sequence and the 16S rDNA sequence are generally used as a species marker among fungi or *Streptomyces*, respectively. In our study, 75% of the isolates were identified at the species level by sequencing their ITS region or the 16S rDNA. Most of the remaining isolates were not identified at the species level because of a too short sequence. However, this does not stand for the isolates belonging to the genus *Alternaria.* Several of them could not be identified at the species level even with an almost complete ITS sequence because their ITS sequence matched two or more *Alternaria* species. In these cases, sequencing of the genes coding the elongation factor, the beta-tubulin, or the mitochondrial ribosomal large subunit (mtLSU) might have been more discriminative (Peever et al. 2004). On the other hand, the variability of ITS sequences is much higher in *R. solani*, allowing the differentiation of AG within the species (Guillemaut et al. 2003). In our study, three different AGs were identified.

The most important one was AG 3 - PT known to be the most frequent AG on potato (Kuninaga et al. 2000). In addition, six isolates of *R. solani* AG 2-1 and four isolates of *R. solani* AG 5 were also isolated. *R. solani* AG 3 - PT and AG 2-1 were mostly isolated from sclerotia whereas *R. solani* AG 5 was isolated from enlarged lenticels and russeting. Similar results were found in potato crops in France and Great-Britain (Campion et al. 2003; Woodhall et al. 2007).

Concerning the identification of *Streptomyces*, identification at the species level has not been possible in all cases. A complementary identification method based on biochemical characteristics of streptomycetes could be used. Otherwise, a specific PCR for each species of *Streptomyces* could allow a quicker and complementary detection. An amplification of the 16S rDNA, highly conserved, and amplification of the ribosomal intergenic spacer (RIS), more discriminative, could allow a better identification of *Streptomyces* species (Lehtonen et al. 2004; Park and Kilbane 2006).

The second part of the study was to assess the pathogenicity of the isolated strains. Two different types of bioassays were set up. In the first one, the soil was artificially infested by the microbial strains in order to mimic field conditions. In the second one, potato plants were grown under hydroponic conditions and inoculated with one or two isolates of potential pathogens, in order to follow the development of the blemishes. Although millet seeds used as inoculum were fully colonized, inoculated isolates were not always detected in the soil at the end of the assay. This could be because the soil volume tested

was too small, or because other species isolated from soil such as *Trichoderma* or *Mucor* having a high growth rate, prevented the growth of the fungi of interest. Among the 72 fungal isolates tested in soil, only 14 were able to reproduce the same blemishes they originated from; the other isolates did not reproduce the blemish of origin. Among the 11 *Streptomyces* isolates tested, none was able to reproduce the type of blemish it originated from. According to Koch's postulates, a given isolate can be considered as responsible for a symptom when it reproduces the original symptom and when the identical isolate can be recovered at least once from the diseased progeny tubers (Rapilly 2001). The 14 potentially pathogenic isolates belonged to the species *F. oxysporum*, *P. brevicompactum, P. cucumerina*, and *R. solani* AG 3 - PT. Skinning, enlarged lenticels and polygonal lesions seemed to be the basal blemishes observed under the conditions of the assay rather than a result of the inoculum. Even though *Fusarium* spp. and more especially *F. oxysporum* were frequently isolated from blemishes and particularly associated with polygonal lesions, it seems that this species is well adapted to live on tuber surface as an opportunist. Because, polygonal lesions were also observed on the non-inoculated controls, it is not possible, at this point, to infer the involvement of this species in the formation of polygonal lesions. Similarly, we cannot consider *P. cucumerina, P. brevicompactum* and *R. solani* AG - PT as responsible for enlarged lenticels on potatoes. We know that a high level of humidity in the soil is favourable for the opening of lenticels as it was observed in the non-inoculated controls of the bioassays. Opened lenticels facilitate penetration of microorganisms. *P. cucumerina, P. brevicompactum* and *R. solani* AG 3 - PT could be well adapted species to this specific niche and might be considered as opportunistic colonizers of the lenticels. Moreover, isolations made from soil from the bioassays showed that some species were better adapted to survive in the soil than others (data not shown). *R. solani* did not spread homogenously into the soil since it was much more often recovered from the tuber surface than from the soil. *Alternaria* spp. and *Clonostachys* spp. were recovered neither from the soil nor from the tuber surface. While *Clonostachys* is known to be good saprophyte (Theron 1991), *Alternaria and R. solani* are not. *R. solani* behaves as a root inhabiting fungus rather than as a soil fungus (Garrett 1970).

The involvement of *R. solani* AG 3 - PT in the formation of polygonal lesions was observed in 4 independent replicates: this result confirms what is currently observed in open field conditions but needs further investigation under controlled conditions and artificial inoculation. Similarly, the contribution of *R. solani* AG 3 - PT to the formation of corky crack was verified with only one isolate. On the contrary, the pathogenicity of *R. solani* AG 3 - PT causing sclerotia was clearly demonstrated in four independent replicates. The involvement of *R. solani* AG 3 in the occurrence of sclerotia has already been demonstrated several times (Anderson 1982; Woodhall et al. 2008).

Since inoculation of single isolates did not allow the reproduction of observed atypical blemishes, we assumed that a consortium of isolates might be involved in the occurrence of blemishes. Co-inoculations, in the bioassay in hydroponic conditions, did not demonstrate clear fungal or *Streptomyces* contribution into the occurrence of skin blemishes. Blemishes like skinning or star-like corky lesions could be the early stage of other blemishes like polygonal lesions. We also found a connection between wound healing and the formation of polygonal lesions. Moreover, the appearance of severe polygonal lesions on the non-inoculated tubers under hydroponic conditions confirmed that this blemish could be skin response to other environmental factors. In the 60 – 70's and later in the 2000's, several publications suggested that polygonal lesions were due to an excess of humidity or organic matter (Hart 1971; Sexton 2003). In our experimental set up, the high moisture content due to hydroponic conditions is probably one of the causes of some of the blemishes. Polygonal lesions could be the result of a physiological reaction of the plant to stressful abiotic factors.

Although, we noticed that cultivar Samba was particularly frequently associated with polygonal lesions, the genetic background of the potato germplasm was deliberately not taken into account in the expression of tuber blemishes because too little reliable data are available on the host-parasite relationships. However, the *S. tuberosum – Streptomyces* spp. complex is one of few that gather practical data at the cultivar level when assessed under controlled conditions and artificial inoculum (Pasco et al. 2005), although its expression can vary because it is highly dependant upon biotic and abiotic environmental factors.

This study showed the diversity of the microbial flora on the surface of potato tubers and demonstrated that, except for *R. solani* AG 3 - PT causing sclerotia, blemishes analyzed in this study could not be directly attributed to specific microorganisms. The evidence indicates that stress factors induce a differential response of the plant and the diversity of the microorganisms associated with the potato could be a natural situation. Cultivar susceptibility is also probably a key to blemish development and thus control. Complementary studies about the environmental conditions responsible for development of potato blemishes and the assessment of susceptible and resistant cultivars are needed.

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References

Anderson, N. A. (1982). The genetics and pathology of *Rhizoctonia solani*. *Annual Review of Phytopathology, 20*, 329–347.

Bouchek-Mechiche, K., Pasco, C., Andrivon, D., & Jouan, B. (2000). Differences in host range, pathogenicity to potato cultivars and response to soil temperature among *Streptomyces* species causing common and netted scab in France. *Plant Pathology, 49*, 3–10.

Braker, G., Ayala-del-Rio, H. L., Devol, A. H., Fesefeldt, A., & Tiedje, J. M. (2001). Community structure of denitrifiers, Bacteria, and Archaea along redox gradients in pacific northwest marine sediments by terminal restriction fragment length polymorphism analysis of amplified nitrite reductase (nirS) and 16S rRNA genes. *Applied and Environmental Microbiology, 67*, 1893–1901.

Campion, C., Chatot, C., Perraton, B., & Andrivon, D. (2003). Anastomosis groups, pathogenicity and sensitivity to fungicides of *Rhizoctonia solani* isolates collected on potato crops in France. *European Journal of Plant Pathology, 109*, 983–992.

Cwalina-Ambroziak, B. (2002). Fungi colonizated of potato tubers (*Solanum tuberosum* L.) after harvest and after storage. *Acta Agrobotanica, 55*, 133–140.

Davide, R. G., & Zorilla, R. A. (1995). Evaluation of three nematophagous fungi and some nematicides for the control of potato cyst nematode *Globodera rostochiensis*. *Biocontrol, 1*, 45–55.

Edwards, U., Rogall, T., Blocker, H., Emde, M., & Bottger, E. C. (1989). Isolation and direct complete nucleotide determination of entire genes - characterization of a gene coding for 16S-ribosomal RNA. *Nucleic Acids Research, 17*, 7843–7853.

El Bakali, A. M., & Martin, M. P. (2006). Black scurf of potato. *Mycologist, 20*, 130–132.

Fiers, M. (2010). Origins of the blemishes of potato tubers: from the soil microbiology to the pedoclimatic environment. Doctorate Thesis, Université de Bourgogne, (Dijon-France).

FNPPPT & GNIS. (2010). Le plant français de pomme de terre [cited 03 31 2010] Retrieved from www.plantdepommedeterre.org.

Gardes, M., & Bruns, T. D. (1993). ITS primers with enhanced specificity for Basidiomycetes - Application to the identification of mycorrhizae and rusts. *Molecular Ecology, 2*, 113–118.

Garrett, S. D. (1970). *Pathogenic root-infecting fungi*. Cambridge: Syndics of the Cambridge University Press.

GNIS & SOC. (2009). Echelle officielle française des maladies du tubercule de pomme de terre [cited 11 24 2009] Retrieved from www.plantdepommedeterre.org.

Gray, D. (1973). The growth of individual tubers. *Potato Research, 16*, 80–84.

Guillemaut, C., Edel-Hermann, V., Camporota, P., Alabouvette, C., Richard-Molard, M., & Steinberg, C. (2003). Typing of anastomosis groups of *Rhizoctonia solani* by restriction analysis of ribosomal DNA. *Canadian Journal of Microbiology, 49*, 556–568.

Hart, T. G. (1971). Fish scale or elephant hide on potatoes. *The vegetarian newsletter, 71*, 5.

Keinath, A. P., Fravel, D. R., & Papavizas, G. C. (1991). Potential of *Gliocladium roseum* for biocontrol of *Verticillium dahliae*. *Phytopathology, 81*, 644–648.

Kuninaga, S., Carling, D. E., Takeuchi, T., & Yokosawa, R. (2000). Comparison of rDNA-ITS sequences between potato and tobacco strains in *Rhizoctonia solani* AG-3. *Journal of General Plant Pathology, 66*, 2–11.

Lambert, D. H., & Loria, R. (1989). *Streptomyces scabies* sp-nov, nom-rev. *International Journal of Systematic Bacteriology, 39*, 387–392.

Lehtonen, M. J., Rantala, H., Kreuze, J. F., Bang, H., Kuisma, L., Koski, P., et al. (2004). Occurrence and survival of potato scab pathogens (*Streptomyces species*) on tuber lesions: quick diagnosis based on a PCR-based assay. *Plant Pathology, 53*, 280–287.

Lutaladio, N., & Castaidi, L. (2009). Potato: The hidden treasure. *Journal of Food Composition and Analysis, 22*, 491–493.

Park, H. S., & Kilbane, J. J. (2006). Rapid detection and high-resolution discrimination of the genus *Streptomyces* based on 16S-23S rDNA spacer region and denaturing gradient gel electrophoresis. *Journal of Industrial Microbiology & Biotechnology, 33*, 289–297.

Pasco, C., Jouan, B., & Andrivon, D. (2005). Resistance of potato genotypes to common and netted scab-causing species of *Streptomyces*. *Plant Pathology, 54*, 383– 392.

Peever, T. L., Su, G., Carpenter-Boggs, L., & Timmer, L. W. (2004). Molecular systematics of citrus-associated *Alternaria* species. *Mycologia, 96*, 119–134.

Pieta, D., & Patkowska, E. (2003). Antagonistic bacteria and fungi limiting potato infection by soil-borne pathogenic fungi. *Journal of Plant Protection Research, 43*, 97–104.

Radtke, W., & Rieckmann, W. (1991). Diseases and pests of the potato. *Maladies et ravageurs de la pomme de terre.*, 168pp.

Rapilly, F. (2001). Champignons des plantes: les premiers agents pathogènes reconnus dans l'histoire des sciences. *C.R. Academie des Sciences de Paris, Science de la vie/ Life Sciences, 324*, 893–898.

Schroers, H. J., Samuels, G. J., Seifert, K. A., & Gams, W. (1999). Classification of the mycoparasite *Gliocladium roseum* in *Clonostachys* as *C. rosea*, its relationship to *Bionectria ochroleuca*, and notes on other *Gliocladium*-like fungi. *Mycologia, 91*, 365–385.

Sexton, P. (2003). Some thoughts on elephant hide. *Spudline*, December.

Stevenson, W. R., Loria, R., Franc, G. D., & Weingartner, D. P. (2001). *Compendium of potato diseases*. St. Paul: The American Phytopathological Society.

Theron, D. J. (1991). Dry rot of potatoes caused by *Gliocladium roseum*. *Plant Pathology, 40*, 302–305.

Wanner, L. A. (2004). Field isolates of *Streptomyces* differ in pathogenicity and virulence on radish. *Plant Disease, 88*, 785–796.

White, T. J., Bruns, T., Lee, S., & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In M. A. Innis, D. H. Gelfand, J. J. Sninsky, & T. J. White (Eds.), *PCR protocols. A guide to methods and applications* (pp. 315–322). San Diego: Academic.

Woodhall, J. W., Lees, A. K., Edwards, S. G., & Jenkinson, P. (2007). Characterization of *Rhizoctonia solani* from potato in Great Britain. *Plant Pathology, 56*, 286–295.

Woodhall, J. W., Lees, A. K., Edwards, S. G., & Jenkinson, P. (2008). Infection of potato by *Rhizoctonia solani*: Effect of anastomosis group. *Plant Pathology, 57*, 897–905.