Identification of Potato Scab Inducing and Suppressive Species of *Streptomyces*

J. M. Lorang, D. Liu, N. A. Anderson, and J. L. Schottel

First, second, and third authors: Department of Plant Pathology; fourth author: Department of Biochemistry, University of Minnesota, St. Paul 55108.

Published as paper No. 20,981 of the contribution series of the Minnesota Agricultural Experiment Station based on research conducted under Project 22-35H, supported by the Red River Valley Potato Growers Association.
Accepted for publication 21 November 1994.

ABSTRACT

Lorang, J. M., Liu, D., Anderson, N. A., and Schottel, J. L. 1995. Identification of potato scab inducing and suppressive species of *Streptomyces*. Phytopathology 85:261-268.

Complementation, co-plating, antibiotic, and taxonomic tests were used to identify pathogenic and suppressive species of *Streptomyces*. Of 17 strains of *Streptomyces* (16 from potato tubers and one from garden beet), 13 were determined to be *S. scabies,* two were *S. diastatochromogenes,* one was *S. albogriseolus,* and one could not be identified. Only the 13 *S. scabies* strains and the one unidentified russet scab strain were pathogenic on leaf-bud tubers. Prototrophs between auxotrophic mutants formed only in intraspecies pairings, and for *S. scabies* at rates of 1.11 and 1.43% for intra- and interstrain pairings, respectively. Tests involving inhibitory reactions of paired strains supported the taxonomic study. In co-plating tests, the type A (lethal zygosislike) reaction occurred only in interspecies pairings. The type B reaction indicated production of and sensitivity to inhibitory compounds. Antibiotic assays confirmed the type B reactions and placed the 17 strains into seven groups that were consistent with the taxonomic work. Three strains, two *S. diastatochromogenes* and one *S. albogriseolus,* obtained from a potato scab plot that had become suppressive, produced antibiotic-like compounds against highly pathogenic strains of *S. scabies*.

Additional keyword: antibiotic biological control.

Potato scab is caused by several gram-positive, filamentous species of *Streptomyces*. These bacteria cause lesions on tuber surfaces and affect quality but have little effect on yield (13). A taxonomic history of the potato scab pathogen *Streptomyces scabies* has been compiled by Lambert and Loria (16). A major problem in *S. scabies* taxonomy arose in 1961 when Waksman (34) redescribed the species but designated IMRU 3018 (= ISP 5078) as the neotype strain. This strain was apparently pathogenic but did not produce spiral spore chains and melanin pigment as described by Thaxter (31). It was unfortunate that this strain represented *S. scabies* in all the International Streptomyces Project (ISP) work and the keys that resulted. Lambert and Loria state that since the 1960s a disproportionate number of putative *S. scabies* reference strains isolated from potato scab lesions have been placed in other species. They (16) also state that, for the above reasons, *S. scabies* was considered an uncertain species in Bergey's Manual of Determinative Bacteriology, 8th ed. However, in 1979 Elesaway and Szabo (7) working in Hungary, recognized the problem and presented a neotype strain of *S. scabies* (strain ATCC 33282). They added to Thaxter's original description the traits of smooth spores and use of all ISP sugars. When the species *S. scabies* did not appear in the Approved Lists of Bacterial Names, Lambert and Loria collected and analyzed 11 strains from common scab lesions on tubers from the eastern U.S., Canada, and Hungary and compared these with other *Streptomyces* species that have been confused with *S. scabies*. They concluded that the majority of pathogenic strains of *Streptomyces* they isolated are a distinct species consistent with the original *S. scabies* description. They recommend that the name *S. scabies* be redesignated to describe this species (16).

In a number of instances, however, potato scab has been reported to be caused by species other than *S. scabies*. Millard and Burr (26) described 11 species of *Streptomyces* that caused scab. The strains that Harrison (10) found to cause a russet scab of tubers in the alkaline Red River Valley soils (pH 8.0) of Minnesota and North Dakota were not identified but were determined not to be *S. scabies*. In the highly acidic soils of northern Maine (pH 5.2 or below) the scab pathogen has been determined to be *S. acidiscabies* (17,23), and in the Pacific Northwest strains causing pit scab of the cultivar Russet Burbank were reported to be caused by six species of *Streptomyces* (1). A recent study in Israel (6) indicated that 57% of the pathogenic strains studied were *S. violaceus* and 22% were *S. griseus*. They found no significant correlation between the groups established by numerical taxonomy and RFLP analysis and that Southern blots on DNA from the pathogenic strains indicated great diversity, suggesting that genes for pathogenicity may reside on mobilizable elements. Healy and Lambert (11) indicated that many strains previously included in *S. scabies* belonged to the *S. albidoflavus* cluster. Their study using DNA-DNA hybridization confirmed phenotypic studies that pathogenic members of *S. scabies, S. acidiscabies,* and *S. albidoflavus* are unrelated.

One way that may indicate genetic relatedness among strains of *Streptomyces* of the same or different species is to note if complementation occurs due to the presence of different auxotrophic mutant nucleoids in a common hypha. The result is limited prototrophic growth on minimal medium and was demonstrated in *S. griseus* and in *S. cyaneus* by Bradley and Lederberg (4). Genetic relatedness of *Streptomyces* species has also been evaluated by studying inhibitory reactions of paired strains. Bibb and Hopwood found that only paired strains having different fertility plasmids formed the lethal zygosis (LZ) reaction (2). In a previous study, *Streptomyces* strains from potato were paired and the LZ-like reaction occurred frequently in pairings of strains from different geographic areas (24). Other inhibitory reactions noted in these studies were due to antibiotic production.

Due to the problems associated with identifying species of *Streptomyces* that cause the scab disease of potato, we used complementation tests, inhibitory reactions, and taxonomic criteria to determine the species and relationship of pathogenic strains of *Streptomyces* as well as of nonpathogenic strains obtained from tubers grown in a soil that became suppressive after 30 yr of potato monoculture.

MATERIALS AND METHODS

Chemicals and Media. Oatmeal agar (OM): 20 g of oatmeal was boiled in 1 L of distilled water for 10 min, strained, and 15 g of agar and 1 g of casamino acid were added. Minimal medium (MM): 35 g of Czapek's-solution agar (Difco) was dissolved in 1 L of distilled water. R2 regeneration medium was made as described by Hopwood et al (14). Tyrosine R2 medium was prepared by adding 1 g of tyrosine to 1 L of R2 regeneration medium. TM buffer contained 0.05 M Tris and 0.05 M maleic acid.

***Streptomyces* isolation and cultivation.** The method used for isolating species of *Streptomyces* from potato scab lesions was that of Harrison (10). When a pure culture was obtained, spore suspensions were made by adding 10 ml of SD water to 7- to 10-day-old OM plates, scraping with a sterile loop, and filtering the resulting suspension through sterile cotton. Spores were harvested by centrifugation at 1,500 rpm for 20 min, resuspended in 20% glycerol, and stored in the freezer at −10 C.

Inoculum for all subsequent experiments was obtained directly from the stored spore suspensions. All strains were cultivated at 30 C on OM or R2 regeneration medium.

Isolation of *Streptomyces* spp. from conducive and suppressive soil. The pathogenic *S. scabies* isolates were obtained from different locations in an 0.8-ha plot on the University of Minnesota Sand Plains Research Station, Becker, MN (Table 1). The plot was established by adding 4 tons of lime ($CaCO_3$) to soil that had never previously been cropped to potatoes. The above isolates were obtained in the thirteenth year of potato monoculture and at that time scab decline was not evident. The weakly pathogenic *S. scabies* strain PonR was also obtained from this plot. The *S. scabies* strain from garden beet was from a neighboring plot (25 m to the north) to which lime had not been added. The *S. scabies* strain (Roy) was isolated from a tuber in a commercial field of cv. Russet Burbank located 64 km north of Becker, MN. Strain Crys, the russet scab strain, was from a tuber of the cv. Crystal grown on the Red River Valley Potato Growers Research Farm, Grand Forks, ND.

Three strains (PonSSI, PonSSII, and PonSSR) were obtained from a potato scab plot in which the disease had declined (Table 1). This plot is located on the University of Minnesota Experiment Station, Grand Rapids, MN. The plot was established in 1942 and potato germ plasm was evaluated for scab resistance each year. In 1965 the disease began to decline, and the plot had to be abandoned in 1972 because the disease did not occur. In 1985–1987, cvs. Cobbler, Pontiac, and Russet Burbank were planted in this plot, two rows of each cultivar, 30.5 m in length. The tubers were harvested each year and no scab was noted. In 1987, isolations were made from slightly raised lenticels of Pontiac and the three strains obtained. An additional strain, PonR, was obtained by isolating from a lenticel of Pontiac grown in our current potato scab plot (Becker, MN) that had been in continuous potatoes for 13 yr (21).

Disease tests. The ability of each strain to cause scab disease was confirmed by inoculating microtubers generated via Lauer's leaf-bud cutting method (18). Five cuttings were placed in each 15-cm clay pot with the stem and axillary buds submerged in moistened sterile sand. After 2–3 wk growth at 18 C in the greenhouse with no supplemental light, microtubers began to form from the axillary buds. The sand was gently brushed away to expose the tubers, and each tuber was inoculated with a 500-μl spore suspension of 10^7–10^8 spores per milliliter. The tubers were covered again with sand for an additional 7–10 days until disease symptoms were visible.

Two pathogenic strains of *S. scabies* were used to compare the type of lesion produced by each on tubers produced by the leaf-bud and seed-piece methods. Plants derived from the single-eye of a tuber were grown in 15-cm pots and transplanted into 12-L plastic pots just prior to flowering. The top 50% of pasteurized soil in the 12-L pots was removed from the pots and thoroughly mixed with inoculum consisting of agar, hyphae, and spores of 4 OM plates of fully sporulating cultures. The infested soil was returned to the pots and scab symptoms were evaluated after 5 wk.

Taxonomic studies. The strains of *Streptomyces* used in this study were identified to species using the criteria of Lambert and Loria (16) for *S. scabies*; Bergey's Manual of Determinative Bacteriology 8th Edition (5) and Shirling and Gottlieb's (30) methods for taxonomic studies on *Streptomyces* species for other species. The type strain of *S. scabies* used in this study was RL 34 (= ATCC 49173) provided by R. Loria.

NTG mutagenesis. Spore suspensions of each strain were centrifuged at 1,500 rpm for 20 min and the spore pellets suspended in 1 ml of TM buffer (pH 9) containing N-methyl-N′-nitro-N-nitrosoguanidine (NTG, 2 mg/ml) for 2 hr at 30 C. NTG was removed by centrifugation and discarded with the supernatant. Spores were washed three times in SD water and resuspended in SD water (14). Percent lethality was determined by subjecting a sample from the original spore suspensions to all the above treatments except exposure to NTG and then plating to determine

TABLE 1. The species, strain designation, host, original lesion type, origin, and disease index on leaf-bud tubers of 17 strains of *Streptomyces*

Species	Strain	Host	Original lesion[a]	Origin	Disease index[b]
S. scabies	FLII	Atlantic	S	Florida	2/3
S. scabies	Beet[c]	Garden Beet	P	Becker, MN	5/4
S. scabies	BC[c]	Cobbler	S	Becker, MN	3/3
S. scabies	Roy[c]	Russet Burbank	P	Royalton, MN	5/4
S. scabies	NC	Norchip	P	Becker, MN	5/4
S. scabies	RB2	Russet Burbank	P	Becker, MN	5/4
S. scabies	RB3	Russet Burbank	P	Becker, MN	5/4
S. scabies	RB3II	Russet Burbank	P	Becker, MN	5/3
S. scabies	RB4	Russet Burbank	S	Becker, MN	5/4
S. scabies	RB5	Russet Burbank	R	Becker, MN	5/4
S. scabies	PonP	Pontiac	P	Becker, MN	5/4
S. scabies	PonC	Pontiac	S	Becker, MN	4/3
S. scabies	PonR	Pontiac	R	Becker, MN	2/3
S. spp.	Crys	Crystal	R	Grand Forks, ND	2/3
S. diastatochromogenes	PonSSI	Pontiac	L	Grand Rapids, MN	0
S. diastatochromogenes	PonSSII	Pontiac	L	Grand Rapids, MN	0
S. albogriseolus	PonSSR	Pontiac	L	Grand Rapids, MN	0

[a] Lesion type designators: P = pit scab; S = superficial common scab; R = russet scab; and L = from lenticels of scab-free tubers grown in suppressive soil.

[b] Disease index values determined by pathogenicity tests on Pontiac and Russet Burbank microtubers. The numerator represents lesion type: 1 = very small, superficial; 2 = small, superficial; 3 = broken periderm; 4 = pit; 5 = deep pit. The denominator represents tuber coverage: 1 = trace, 2 = light; 3 = medium; 4 = heavy.

[c] Pathogenicity determined on Kennebec only.

the viable spore concentration; 99.5% lethality was usually obtained.

Mutagenized spore suspensions were plated (300 μl per plate) on OM and allowed to grow for 7–10 days at 30 C. Spores were harvested from these plates and stored as previously described.

Mutant isolations. Auxotrophic mutants were isolated by replica plating (19) sporulating colonies of NTG-treated spores (approximately 200 per plate) from complete medium (OM) to minimal medium (MM). Putative mutants were picked and replated on OM several times to insure stability and adequate growth. Mutants were characterized by plating on differential media (12) supplemented with growth factors at a concentration of 20 mg/L for amino acids and 2 mg/L for vitamins. Approximately 0.05% of viable colonies were mutant for some growth factor.

Complementation tests. Methods for pairing auxotrophic mutant strains were described previously (3,4,9). Two mutant spore suspensions, a 50-μl aliquot of each (10^5 spores per milliliter), were co-plated on MM. Because some mutants were "leaky" and produced a light background of substrate mycelium, 100 μl of each parent was also plated separately on MM. These single strain plates were compared with the plates containing two strains when evaluating presumptive prototrophic growth due to complementation. The mutants were paired (Table 2) in all possible combinations and repeated at least twice in separate experiments. Those pairings yielding aerial mycelium or increased growth over the parental types were repeated a third time.

To differentiate between growth due to syntrophism (cross-feeding) and that due to complementation or recombination, mutant strains were plated on opposite sides of a cellulose membrane (Spectrapor, M.W. cutoff 12,000 Da, Spectrum Medical Industries, Inc., Los Angeles, CA). Paired mutants that grew due to syntrophism through the membrane were considered not to have complemented even though complementation and syntrophism could have occurred simultaneously. Pairings yielding protrophic growth in the absence of syntrophism were designated putative prototrophs or recombinants. Nine of these were selected to determine if both parental types could be recovered from a single hyphal fragment obtained by micromanipulation (4). The single hyphal tip or single hyphal fragments were grown for 2–3 days in an OM-broth hanging drop and then transferred to an OM-agar plate and allowed to sporulate. Spores were harvested and screened by replica plating onto MM, and the differential medium (12) used to determine mutant phenotype as previously described.

Co-plating reactions. Growth inhibition assays were similar to those of McQueen et al (24). Strains were tested for their ability to elicit lethal zygosislike reactions or to produce antibiotics by growing patches (2 cm diameter) on OM agar until fully sporulating. To be tested for susceptibility to inhibitory reactions, these patches were replica plated onto R2 regeneration medium freshly seeded with lawns of strains made with spores stored at −10 C. After 3–5 days incubation at 30 C, LZ-like reactions (type A), antibiotic-like inhibitions (type B), or no visible antagonism (type C) reactions were observed.

Antibiotic assays. Antibiotic assays were made using the double-layer agar method of Vidaver et al (33). Test strains were plated (10 μl at 10^5 spores per milliliter) 4–5 spots per plate on R2 regeneration medium (20 ml/75 mm plate) and incubated at 30 C for 3 days. The bacteria were then killed by inverting each plate over 3 ml of chloroform for 1 hr. The plates were left with the lids ajar for an additional hour until all chloroform had evaporated, and then overlaid with 15 ml of 1% water agar that upon hardening was seeded with 100 μl of a test strain containing 10^5 spores per milliliter. The plates were inverted and incubated at 30 C for 3–5 days. Zones of growth inhibition were observed in the lawns when antibiotics were produced by the test strains.

TABLE 2. Auxotrophic mutant strains of *Streptomyces scabies, S. diastatochromogenes* and an unidentified strain of *Streptomyces* causing a "russet scab" of potato

Designation[a]	Phenotype[b]	Designation[a]	Phenotype[b]
Streptomyces scabies		RB4-2	vit
FLII-1	–	RB5-1	his
FLII-2	nia	RB5-2	nia
FLII-3	–	RB5-3	his
FLII-4	met	PonP-1	pyr
Beet-1	his	PonP-2	thi
Beet-2	pyr	PonC-1	lys
Beet-3	met	PonC-2	–
Beet-4	met	PonC-3	–
Beet-5	his	PonC-4	vit
BC-1	pyr	PonR-1	–
BC-2	met	PonR-2	–
BC-3	–	*Streptomyces diastatochromogenes*	
BC-4	vit	PonSSI-1	met
BC-5	nia	PonSSI-2	met
Roy-1	met	PonSSI-3	met
Roy-2	his	PonSSI-4	met
Roy-3	leu	PonSSI-5	–
Roy-4	met	PonSSI-6	met
Roy-5	–	PonSSII-1	–
NC-1	leu	PonSSII-2	met
NC-2	–	PonSSII-3	met
RB2-1	met	PonSSII-4	–
RB3II-1	–	*Streptomyces* spp.	
RB3II-2	NH_4[c]	"Russet scab" strains	
RB3II-3	–	Crys-1	thi
RB3II-4	NH_4[c]	Crys-2	vit
RB3II-5	–	Crys-3	–
RB4-1	–		

[a] Mutant strains are designated by name of strain from which they were derived, followed by a number (1–5) to differentiate between strains.

[b] Phenotypes are named by abbreviation of the growth factor that will restore growth of strain when added to minimal medium: his = histidine, leu = leucine, lys = lysine, met = methionine, NH_4 = ammonium, nia = niacinamide, pyr = pyridoxal hydrochloride, pyrdoxamine dihydrochloride, or pyridoxine hydrochloride, thi = thiamine hydrochloride, and vit = a vitamin solution, exact phenotype unknown. (–) signifies strains that grow on oatmeal agar medium but not on minimal medium and phenotype is unknown.

[c] Signifies that the given nutrient increases growth slightly, but exact phenotype is unknown.

RESULTS

The geographic origin, host, original lesion type, disease index on leaf-bud tubers, and species of the 17 strains of *Streptomyces* used in this study are presented in Table 1. Thirteen of these strains (12 from potato, one from garden beet, *Beta vulgaris*) were pathogenic on leaf-bud tubers, had smooth gray spores that formed in spiral chains, produced a melanin pigment on tyrosine agar, utilized all the ISP sugars, had a minimum growth pH of 5.0, and were identified as *S. scabies* (20, Table 3).

One strain (Crys) produced russet scab lesions on leaf-bud tubers and could not be identified in the taxonomic tests (Table 3). This strain is similar to the russet scab strains described but not taxonomically identified by Harrison (10). Two strains (PonSSI and PonSSII) obtained from lenticels of scab-free tubers (cv. Pontiac) grown in the scab-suppressive soil were nonpathogenic on leaf-bud tubers and were identified as *Streptomyces diastatochromogenes* (15,20). A third strain (PonSSR), also obtained from scab-free tubers (cv. Pontiac) grown in the suppressive-soil plot, was nonpathogenic on leaf-bud tubers and was identified as *Streptomyces albogriseolus* (Table 3).

None of the *S. scabies* strains obtained from potato were cultivar specific and all strains produced similar types of lesions on leaf-bud tubers of cvs. Kennebec and Russet Burbank (Fig. 1). The *S. scabies* strain from table beet caused pit lesions on microtubers of Kennebec. All strains obtained from Russet Burbank produced deep pit lesions even though strains RB4 and RB5 were originally obtained from superficial lesions. With the exception of these two strains, the other strains produced lesions on the leaf-bud tubers that were similar to those lesions from which they were obtained. Strains PonC and RB311 were also tested on tubers of whole plants. Strain PonC, originally isolated from a type 3 lesion on a Pontiac tuber, produced type 3 lesions on the leaf-bud tubers and on 8 of 17 Russet Burbank tubers. Strain RB311 obtained from a deep pit lesion (type 5) on a Russset Burbank

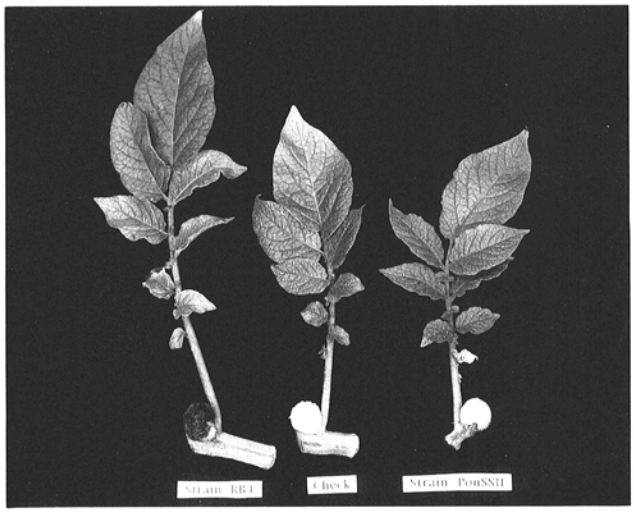

Fig. 1. Potato leaf-bud tubers cv. Kennebec inoculated with virulent strain RB4 (left), with strain PonSSII from disease suppressive soil (right), and water control (center).

TABLE 3. Characteristics of *Streptomyces scabies* and other *Streptomyces* species and strains[a]

	Strains of *S. scabies*						Strains of *S. scabies*								*S. diastatochromogenes*		*S. albo-griseolus*
	FLII	Beet	BC	Roy	NC	Crys	RB2	RB3	RB3II	RB4	RB5	PonP	PonC	PonR	PonSSI	PonSSII	PonSSR
Chain morphology[b]	S	S	S	S	S	RF	S	S	S	S	S	S	S	S	RF	RF	S
Melanin on tyrosine agar	+[c]	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
Pigment on PYI[d]	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
Diffusible pigment	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Carbon usage																	
L-Arabinose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-Fructose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-Mannitol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	±	±	±
Raffinose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Rhamnose	+	+	+	+	+	+	+	+	±	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-Xylose	+	+	±	+	+	+	+	+	+	+	+	+	+	+	+	+	+
meso-Inositol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-Galactose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salicin	±	±	±	±	±	±	±	±	±	±	±	±	±	+	±	±	+
Nitrogen usage																	
L-Hydroxyproline	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+
L-Methionine	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
Degradation of:																	
Arbutin	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polygalacturonate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Xanthine	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Xylan	+	+	+	+	+	−	+	+	+	+	+	+	+	+	−	−	+
Minimum growth pH	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	4.5
Growth with:																	
5% NaCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6% NaCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
7% NaCl	−	+	+	−	+	+	+	+	+	+	+	+	+	−	+	+	−
Tellurite (10 μg/ml)	+	+	+	−	+	+	+	+	+	+	+	+	+	−	+	+	−
Tellurite (100 μg/ml)	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−
Thallium (10 μg/ml)	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−
Thallium (100 μg/ml)	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Crystal violet (0.5 μg/ml)	−	−	−	−	−	−	−	−	−	+	−	−	−	−	+	+	−
Phenol (0.1%)	−	+	+	−	+	+	−	+	+	+	+	−	−	+	−	−	+
Penicillin (10 IU/ml)	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−
Oleandomycin (25 μg/ml)	+	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−
Oleandomycin (100 μg/ml)	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Streptomycin (20 μg/ml)	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−

[a]For all *Streptomyces* spp. and strains given, spore color is gray and spore ornamentation is smooth.
[b]S, spiral; RF, rectiflexuous.
[c]+, positive reaction; −, negative reaction.
[d]Peptone yeast extract iron agar.

tuber, produced type 5 lesions on the leaf-bud tubers and on 14 of 15 Pontiac tubers.

Complementation tests. A total of 53 mutant strains generated by NTG mutagenesis were used in this study. Forty mutant *S. scabies* strains were obtained; 27 strains had their auxotrophy determined, but the nutritional requirement of 13 strains could not be determined. Three mutant "russet scab" strains (Crys) of the unknown species of *Streptomyces* were also obtained, two were auxotrophs and one could not be determined. Ten mutant strains of *S. diastatochromogenes* were also obtained, seven were auxotrophs and the nutritional requirement of three strains was not determined. Many mutants that were characterized initially were not stable, and numerous other mutants had apparently complex nutritional requirements that could not be identified by the method used. These mutants were not used in these tests. In this work, only stable mutants that did not grow on MM but did grow well on OM were kept and used in the study even though the specific auxotrophic phenotypes of some were unknown (Table 2).

The 53 mutant strains of *Streptomyces* were paired in all possible combinations (1,378 pairings). Of these pairings, 210 resulted in growth on MM due to syntrophism and these were therefore eliminated from the study. Complementation and prototrophic growth occurred only among intraspecies pairings of mutant strains of *S. scabies, S. diastatochromogenes,* and the unidentified mutant strains of *Streptomyces* that caused russet scab (Fig. 2). From a total of 40 *S. scabies* mutant strains, 18 complemented in at least one pairing. The results of pairing these 18 mutant *S. scabies* strains in all combinations are presented in Table 4 and indicate that complementation and prototrophic growth occurred on MM in nine interstrain and 10 intrastrain pairings. Three intrastrain prototrophs formed in pairings between the three *S. scabies* mutant strains from Florida but no prototrophs formed in pairings between these three Florida mutants and any mutant *S. scabies* strains from Minnesota.

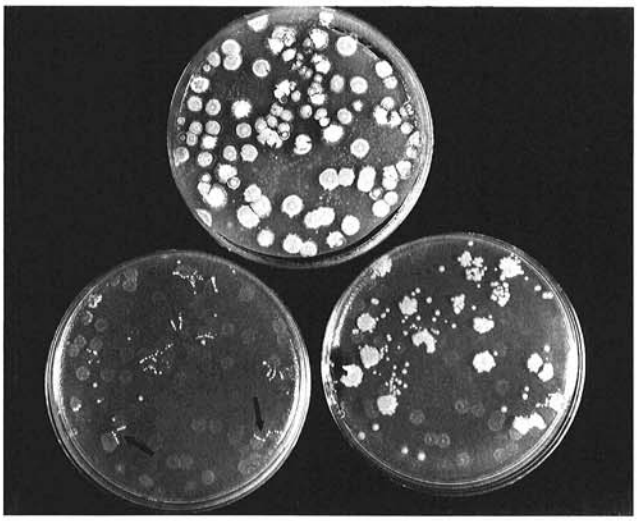

Fig. 2. Complementation test. Masterplate of sporulating colonies of a putative prototroph grown on oatmeal agar medium (top) was replica plated onto: (1) minimal medium supplemented with the nutritional requirement of one parent of the prototroph (bottom right, showing presence of two nutritional mutants); (2) minimal medium, prototroph forms (arrows) at junction of the two nutritional mutants (bottom left).

Four intrastrain *S. scabies* prototrophs formed in pairings between the four mutant strains (ROY1, ROY2, ROY3, ROY4) isolated from pit scab lesions on Russet Burbank tubers near Royalton, MN, and two interstrain prototrophs formed in pairings between mutant strains ROY1 and ROY3 and mutant strain RB311, also isolated from a pit scab lesion on Russet Burbank potatoes, but from a field at Becker, MN, 64 km from Royalton, MN. No complementation and prototrophic growth occurred in pairings between the mutant strains causing pit scab on Russet Burbank and those causing common scab on cvs. Cobbler, Norchip, and Pontiac. Of the remaining 10 *S. scabies* prototrophs that formed, seven were the result of interstrain pairings and three were from intrastrain pairings.

Eight mutants derived from *S. diastatochromogenes* strains PonSSI and PonSSII from the scab-suppressive soil were paired in all possible combinations on MM (Table 5). No intrastrain prototrophs formed in pairings of five PonSSI strains mutant for methionine and this suggests these mutants may be allelic. In the $PonSSII_7$ and $PonSSII_8$ intrastrain pairings, prototrophic growth developed, indicating these methionine requiring mutants were not allelic. Nine prototrophs formed in the interstrain pairings, suggesting that the five PonSSI methionine mutants differed from the two different methionine-requiring PonSSII mutants. The uncharacterized PonSSII mutant did not complement in any pairing. No interspecies complementation was observed in any pairings involving the *S. diastatochromogenes* mutants (data not shown).

An intrastrain prototroph formed in the pairing of a thiamin and an undetermined vitamin mutant of the russet scab strains (Crys) from North Dakota. Again, no interspecies complementation was observed in any pairings involving the russet scab mutant strains (data not shown).

Hyphal fragments or hyphal tips were obtained by micromanipulation from nine pairings of auxotrophic mutants that

TABLE 4. Prototrophs resulting from the pairing of 18 mutant strains of *Streptomyces scabies*

Mutant strains	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1. Fl-1$^-$	—[a]	⊕[b]	⊕	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
2. Fl-2^{nia}		—	⊕	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
3. Fl-3$^-$			—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
4. BC-1^{pyr}				—	—	—	—	—	—	—	+[c]	—	—	—	+	—	—	—
5. BC-2^{met}					—	—	—	—	—	—	—	+	—	—	+	—	—	—
6. BC-3$^-$						—	—	—	—	—	+	—	—	—	—	—	—	—
7. Roy-1^{met}							—	⊕	—	—	—	—	+	—	—	—	—	—
8. Roy-2^{his}								—	⊕	⊕	—	—	—	—	—	—	—	—
9. Roy-3^{leu}									—	⊕	—	—	+	—	—	—	—	—
10. Roy-4^{met}										—	—	—	—	—	—	—	—	—
11. NC-1^{leu}											—	⊕	—	—	—	—	—	—
12. NC-2$^-$												—	—	—	+	—	—	—
13. RB311^{NH4}													—	—	—	—	—	—
14. PonP-1^{pyr}														—	⊕	—	—	—
15. PonP-2^{thi}															—	—	+	—
16. PonC-2$^-$																—	—	⊕
17. PonC-3$^-$																	—	—
18. PonC-4^{vit}																		—

[a] No complementation.
[b] Intrastrain prototroph.
[c] Interstrain prototroph.

resulted in complementation and prototrophic growth on MM. From each of the nine prototrophs, only the two parental mutant-type spores were recovered. For two prototrophs, up to 10,000 spores were tested and no prototrophs were found. These results indicated that complementation was not the result of recombination.

Inhibitory reactions. All 17 strains (Table 1) were co-plated against each other in all combinations to evaluate inhibitory reactions among strains and thus their competitive ability (Table 6). Three types of interactions were observed. The type A reaction describes a thin inhibitory zone between paired strains. This reaction is typical of the lethal zygosis reaction described by Bibb and Hopwood (2). It also resembles the "killing reaction" of filamentous fungi, particularly the recognition interaction of two field isolates that belong to the same anastomosis group of *Thanatephorus cucumeris* (*Rhizoctonia solani*) (27). The type A reaction was evident when strain Crys (russet scab strain, *Streptomyces* spp.) was replica plated onto lawns of all the other strains in this study except strain FL11.

The type B reaction has a growth inhibition zone between the two colonies and appears to be due to a diffusible substance(s). The three strains obtained from the suppressive-soil plot (PonSSI, PonSSII, and PonSSR) produced this type of reaction when replicated on lawns of all the *S. scabies* strains. The type C reaction suggests no visible antagonism of one strain against the other. This reaction was typical of a strain paired with itself and of all the highly pathogenic *S. scabies* strains from Minnesota that were paired with each other. The two *S. diastatochromogenes* PonSSI and PonSSII strains from the suppressive-soil plot also gave the type C reaction when co-plated with each other but, when paired with *S. albogriseolus* strain PonSSR from the same soil, gave the A reaction if PonSSR was the patch and the B reaction if PonSSI or PonSSII was the patch.

Antibiotic assays. The 17 strains were paired in all possible combinations to appraise their ability to produce antibiotics and to observe their sensitivity to those produced by other strains. The zones of inhibition in the lawn of a test strain were designated weak (W), medium (M), or strong (S) according to the diameter of the inhibition zone. The 17 strains were placed into seven groups based on their antibiotic-producing ability and sensitivity to antibiotics of other strains (Table 7). The largest group (group 1) consisted of nine highly virulent strains of *S. scabies* from Minnesota, and none of these strains produced visible zones of inhibition against each other. The two strains composing group 2

TABLE 5. Prototrophs resulting from the pairing of eight mutant strains of *Streptomyces diastatochromogenes*

Mutant strains	1	2	3	4	5	6	7	8
1. PonSSI-1^{met}	—[a]	—	—	—	—	—	—	+[b]
2. PonSSI-2^{met}		—	—	—	—	—	+	+
3. PonSSI-3^{met}			—	—	—	—	+	+
4. PonSSI-4^{met}				—	—	—	+	+
5. PonSSI-6^{met}					—	—	+	+
6. PonSSII-1^{-}						—	—	—
7. PonSSII-3^{met}							—	⊕[c]
8. PonSSII-4^{met}								—

[a]No complementation.
[b]Interstrain prototroph.
[c]Intrastrain prototroph.

TABLE 6. Inhibitory reactions among *Streptomyces* strains used in this study in co-plating tests

	Patch[a]																
Lawn	FLII	Beet	BC	Roy	NC	RB2	RB3	RB3II	RB4	RB5	PonP	PonC	PonR	Crys	PonSSI	PonSSII	PonSSR
FLII	C[b]	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
Beet	B	C	C	C	C	C	C	C	C	C	C	C	C	A	B	B	B
BC	B	C	C	C	C	C	C	C	C	C	C	C	C	A	B	B	B
Roy	B	C	C	C	C	C	C	C	C	C	C	C	C	A	B	B	B
NC	B	C	C	C	C	C	C	C	C	C	C	C	C	A	B	B	B
RB2	B	C	C	*[c]	*	C	C	C	C	C	C	C	C	A	B	B	B
RB3	B	C	C	C	C	C	C	C	C	C	C	C	C	A	B	B	B
RB3II	B	C	C	C	C	C	C	C	C	C	C	C	C	A	B	B	B
RB4	B	C	C	C	C	C	C	C	C	C	C	C	C	A	B	B	B
RB5	B	C	C	C	C	C	C	C	C	C	C	C	C	A	B	B	B
PonP	B	C	C	C	C	C	C	C	C	C	C	C	C	A	B	B	B
PonC	B	C	C	C	C	C	C	C	C	C	C	C	C	A	B	B	B
PonR	B—[d]	B—	B—	B—	B—	B—	B—	B—	B—	B—	B—	B—	C	A	B	B	B
Crys	B	B	B	B	B	B	B	B	B	B	B	B	C	C	B	B	B
PonSSI	B	C	C	C	C	C	C	C	C	C	C	C	C	A	C	C	A
PonSSII	B	C	C	C	C	C	C	C	C	C	C	C	C	A	C	C	A
PonSSR	B	A	A	A	A	A	A	A	A	A	A	A	C	A	B	B	C

[a]Sporulating patches of strains across the top were replicated onto freshly seeded lawns of strains listed on the left.
[b]Inhibitory reactions: A = a lethal zygosislike reaction; B = an antibiotic reaction; C = a compatible reaction.
[c]Represents the inconsistent type B reaction alternating with a type C.
[d](—) signifies a weak reaction.

TABLE 7. The 17 parent strains of *Streptomyces* in this study placed in seven groups based on their production and sensitivity to antibiotics in antibiotic assays

Spot		Lawn						
Species	Groups	Roy, NC, Beet, BC, RB2, RB4, RB5, PonP, PonC 1	RB3, RB3II 2	PonR 3	FLII 4	Crys "Russet Scab" 5	PonSSI, PonSSII 6	PonSSR 7
S. scabies	1	...[a]	...	...	...	M[b]	...	...
S. scabies	2	...	...	...	...	M	...	...
S. scabies	3	S	W–M	...	S	S	M	S
S. scabies	4	W[c]	W[c]	...	...	...	...	...
S. spp. "russet scab"	5	W	W	W	...	...	W–M	M
S. diastatochromogenes	6	S	W–M	...	M	...	...	M
S. albogriseolus	7	M–S	M	M	M	S	S	...

[a]No visible inhibitory reaction.
[b]The diameters of the inhibition zones produced in the lawns: weak (W) < 12 mm; medium (M) 12–20 mm; and strong (S) >20 mm (up to 35 mm).
[c]Inconsistent reaction, not always repeatable.

were also highly virulent *S. scabies* strains but differed from the group 1 strains by showing slightly more resistance to the antibiotics produced by strains in groups 3, 6, and 7. However, groups 1 and 2 did produce antibiotics when spotted on lawns of the Russet scab strain (Crys). Strains PonSSI and PonSSII obtained from the suppressive scab plot and identified as *S. diastatochromogenes* were placed in group 6 (Fig. 3). Strains in group 6 produced antibiotics against strains in both groups 1 and 2, against PonSSR the group 7 strain, and the *S. scabies* strain from Florida placed in group 4. The group 3 strain, *S. scabies* PonR, a low-virulence strain, and the strain representing group 7, *S. albogriseolus* strain PonSSR from the suppressive-soil plot, showed a broad activity by producing antibiotics against all the other six groups. The russet scab strain, Crys, group 5, had a unique pattern of antibiotic activity against all groups except group 4. The *S. scabies* strain FLII (group 4) from Florida produced antibiotics only against the highly virulent *S. scabies* strains in groups 1 and 2 from Minnesota.

DISCUSSION

The results of biological tests confirmed the identification of pathogenic strains of *Streptomyces* as well as nonpathogenic strains obtained from a scab-suppressive soil.

Sixteen strains of *Streptomyces* were obtained from potato tubers plus one strain from garden beet, and their pathogenicity, taxonomy, ability of auxotrophic mutants to complement and form prototrophs, inhibitory reactions, and antibiotic production were studied. Ten strains from infected tubers growing in Minnesota potato fields plus one strain from garden beet were highly virulent on leaf-bud tubers and, based on the taxonomic scheme of Lambert and Loria (16), were determined to be *S. scabies*. Two additional strains of low virulence, one from Minnesota and the other from Florida, were also determined to be *S. scabies*. The strain causing russet scab (Crys) could not be identified to species. Russet scab-inducing strains from eastern Canada were recently identified using the Williams et al system of numerical classification of *Streptomyces* (35). These strains were found to have a 78% level of homology with, and were thus included in, the *S. aureofasciens* cluster (8). Two strains (PonSSI and PonSSII) obtained from a potato scab plot that had been abandoned because of disease decline were determined to be *Streptomyces diastatochromogenes*. A third strain (PonSSR) from this suppressive-soil plot was identified as *Streptomyces albogriseolus*.

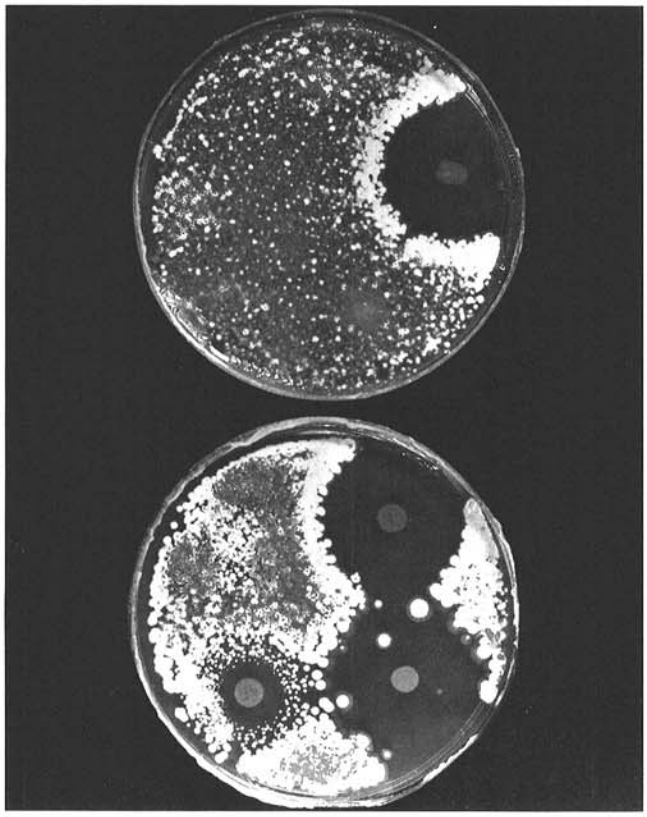

Fig. 3. Antibiotic test showing inhibition of pathogenic *Streptomyces scabies* strain RB3 by strains PonSSR (top), PonSSII (bottom upper right), PonSSI (bottom lower right) and PonR (bottom lower left).

The leaf-bud tuber test was a quick and convenient assay for determining strain virulence. The types of lesions produced by strains on the leaf-bud tubers in the greenhouse were fairly consistent with those from which the strains were originally isolated. Also, lesion types produced by a strain on leaf-bud tubers and tubers of single-eye plants were similar. These results confirm the work of Loria and Kempter (22) that these tests have the potential to aid in screening potato germ plasm for scab resistance.

In this study, results of the complementation tests agreed with those from the taxonomic work. Prototrophic growth on MM is thought to have occurred due to hyphal fusion and complementation between auxotrophic nucleoids in a coenocytic mycelium, and this only occurred in pairings of mutant strains of the same species. However, complementation (heterokaryosis) has been reported in pairings involving different species of *Streptomyces* (4,28). No recombinants were noted in up to 10^4 spores for two prototrophs tested, and only the two parental types were recovered from all the other prototrophs obtained in this work. Syntrophism cannot be completely eliminated as the cause of prototrophic growth on MM. Molecules greater than 12,000 Da of paired mutant strains would allow growth in the absence but not in the presence of a membrane. Molecules of paired strains with a molecular weight less than 12,000 Da could cross the membrane and allow syntrophism to occur. However, if this happened, it only occurred in pairings of the same species.

The complementation test also indicated possible differences in pathogenic strains of *S. scabies* from Minnesota. Complementation did not occur in pairings between strains causing pit scab of Russet Burbank and those causing common scab of tubers of white and red cultivars. However, complementation did occur in both intra- and interstrain pairings among the pit scab strains and among the common scab strains. This study suggests that these strains of *S. scabies* may be genetically isolated.

The frequency of complementation between 18 *S. scabies* auxotrophic mutants paired in all possible combinations (153) was 12.42%. If the three Florida auxotrophs are removed from this diallel leaving only the 15 Minnesota mutant strains, complementation and prototrophic growth occurred in 16 of the 105 possible pairings. Of this 15.24%, 6.67% were intra- and 8.57% were interstrain prototrophs. These estimates of complementation are inflated because, of the 40 *S. scabies* auxotrophic mutants initially paired in all possible combinations, only 18 mutant strains complemented in one or more pairings. The percent complementation and prototrophic growth that formed when all 40 *S. scabies* mutants were paired was $19/780 = 2.44\%$. If one removes the three Florida auxotrophs from the pairings, the percent prototrophs formed was $16/630 = 2.54\%$, of which 1.11% were due to intra- and 1.43% were interstrain pairings. Complementation of auxotrophic nucleoids from different strains in a common cytoplasm appears to indicate genetic relatedness. Its frequency in nature and its significance in relation to genetic recombination need further study.

This study has confirmed the earlier findings of McQueen et al (24) that inhibitory reactions occur between natural strains of *Streptomyces*. The two inhibitory reactions studied were those that appear to require hyphal contact and those due to diffusible antibiotics. In this study, the inhibitory reaction that apparently requires cell contact and produces a thin inhibition zone between the paired strains was consistent with the LZ reactions described by Bibb and Hopwood (2). In our studies, the *S. albogriseolus* strain PonSSR was subject to a LZ-like reaction in interspecific pairings with all *S. scabies* strains from Minnesota. Similarly, the Minnesota *S. scabies* strains, the *S. diastatochromogenes* strains PonSSI and PonSSII, and the *S. albogriseolus* strain PonSSR were all subject to an LZ-like reaction when paired with

the russet scab strain Crys.

The co-plating tests also gave an indication of those strains that had antibiotic-producing ability. Three strains from tubers growing in a suppressive plot (PonSSI, PonSSII, PonSSR) plus a low-virulence strain of *S. scabies* from scab-conducive soil (PonR) produced zones of inhibition when paired with most of the other strains, suggesting antibiotics were involved in this test. The test also gives some indication of the relative competitive ability of two strains and also the possibility of using different suppressive antibiotic producing strains in combination in biocontrol studies. In this test, it appears that antibiotic-producing *S. diastatochromogenes* strains PonSSI or PonSSII would not be effective if paired with *S. albogriseolus* strain PonSSR, as these strains (PonSSI and PonSSII) produce an antibiotic(s) against PonSSR.

It appears that in some pairings the antibiotic or type B reaction can block the expression of the LZ-like type A reaction. This could have happened in the PonSSR:FLII and Crys:FLII pairings.

A total of 17 strains of *Streptomyces* were paired in antibiotic tests in all combinations. The results of these tests, as interpreted by antibiotic production and sensitivity to the antibiotic, allowed us to place the strains into seven groups. Groups 1 and 2 consisted of highly virulent strains identified as *S. scabies,* and none of them produced antibiotics against each other. There were two low-virulence strains of *S. scabies* represented by groups 3 and 4 (from Minnesota and Florida, respectively) that did produce antibiotics against the two strongly virulent *S. scabies* groups 1 and 2, and the Minnesota strain also produced a antibiotic against the Florida strain. The relationship between low virulence and antibiotic production in a population of *S. scabies* needs further study.

Three strains, *S. diastatochromogenes* strains PonSSI and PonSSII and *S. albogriseolus* strain PonSSR, were obtained from a potato scab plot that had become suppressive after 30 yr of potato monoculture (21). The two *S. diastatochromogenes* strains produced antibiotics against all the virulent *S. scabies* strains, against the weakly virulent *S. scabies* Florida strain, and also the *S. albogriseolus* strain PonSSR from the suppressive plot. The *S. albogriseolus* strain PonSSR produced antibiotics against all other strains tested except itself. The discovery of strains from a scab-suppressive soil that produce antibiotics against virulent *S. scabies* strains may be a part of the "biological factor" that Menzies described as responsible for scab decline in a potato scab plot in 1959 (25,29).

Soil from the scab-suppressive plot was added to our current scab-conducive plot used to screen for scab resistance, and the scab disease intensity was reduced (20,21). Scab disease has also been reduced on radish in greenhouse tests using pure cultures of *S. scabies* and *S. diastatochromogenes* strain PonSSII (21). Experiments are being conducted to test the biocontrol potential of these suppressive strains singly and in combination in field tests under natural conditions. To prevent the buildup of antibiotic resistance, Vidaver has suggested that several strains should be used in combination in field tests (32). The inhibition and antibiotic tests indicate the combinations of suppressive strains that would be compatible and that could potentially be used in biological control of the potato scab disease.

LITERATURE CITED

1. Archuleta, J. G., and Easton, G. D. 1981. The cause of deep pitted scab of potatoes. Am. Potato J. 58:385-392.
2. Bibb, M. J., and Hopwood, D. A. 1981. Genetic studies of the fertility plasmid SCP2 and its SCP2* variants in *Streptomyces coelicolor* A3 (2). J. Gen. Microbiol. 126:427-442.
3. Bradley, S. G., and Anderson, D. L. 1958. Compatibility system controlling heterokaryon formation in *Streptomyces coelicolor*. Proc. Soc. Exp. Biol. Med. 99:476-478.
4. Bradley, S. G., and Lederberg, J. 1956. Heterokaryosis in *Streptomyces*. J. Bacteriol. 72:219-225.
5. Buchanan, R. E., and Gibbons, N. E. 1974. Bergey's Manual of Determinitive Bacteriology, 8th ed. Williams and Wilkins Co., Baltimore, MD.
6. Doering-Saad, C., Kämpfer, P., Manulis, S., Kritzman, G., Schneider, J., Zakrzewska-Czerwinska, J., Schrempf, H., and Barash, I. 1992. Diversity among *Streptomyces* strains causing potato scab. Appl. Environ. Microbiol. 58:3932-3940.
7. Elesaway, A. A., and Szabo, J. M. 1979. Isolation and characterization of *Streptomyces scabies* strains from scab lesions of potato tubers. Designation of the neotype strain of *Streptomyces scabies*. Acta Microbiol. Acad. Sci. Hung. 26:311-320.
8. Faucher, E., Otrysko, B., Paradis, E., Hodge, N. C., Stall, R. E., and Beaulieu, C. 1993. Characterization of streptomyces causing russet scab in Quebec. Plant Dis. 77:1217-1220.
9. Gregory, K. G. 1956. Hyphal anastomosis and cytological aspects of *Streptomyces scabies*. Can. J. Microbiol. 2:649-655.
10. Harrison, M. D. 1962. Potato russet scab, its cause and factors affecting its development. Am. Potato J. 39:368-387.
11. Healy, F. G., and Lambert, D. H. 1991. Relationships among *Streptomyces* spp. causing potato scab. Int. J. Syst. Bacteriol. 41:479-482.
12. Holiday, R. 1956. A new method for the identification of biochemical mutants of micro-organisms. Nature 178:987.
13. Hooker, W. J. 1981. Pages 33-34 in: Compendium of Potato Diseases. W. J. Hooker, ed. American Phytopathological Society, St. Paul, MN.
14. Hopwood, D. A., Bibb, M. J., Chater, K. F., Kieser, T., Bruton, C. J., Kieser, H. M., Lydiate, D. J., Smith, C. P., Ward, J. M., and Schrempf, H. 1985. Pages 40-41 in: Genetic Manipulations of *Streptomyces*. A Laboratory Manual. John Innes Foundation, Norwich, CT.
15. Hütter, R. 1967. Systematic der Streptomyceten. Karger, Basel.
16. Lambert, D. H., and Loria, R. 1989. *Streptomyces scabies* sp. nov., nom. rev. Int. J. Syst. Bacteriol. 39:387-392.
17. Lambert, D. H., and Loria, R. 1989. *Streptomyces acidiscabies* sp. nov. Int. J. Syst. Bacteriol. 39:393-396.
18. Lauer, F. I. 1977. Tubers from leaf-bud cuttings: A tool for potato seed certification and breeding programs. Am. Potato J. 54:457-464.
19. Lederberg, J., and Lederberg, E. M. 1952. Replica plating and indirect selection of bacterial mutants. J. Bacteriol. 63:399-406.
20. Liu, D. 1992. Biological control of *Streptomyces scabies* and other plant pathogens. Ph.D. thesis. University of Minnesota, St. Paul, MN.
21. Lorang, J. M. 1988. Heterokaryosis and inhibitory reactions among isolates of *Streptomyces scabies* causing scab on potato. M.S. thesis. University of Minnesota, St. Paul, MN.
22. Loria, R., and Kempter, B. A. 1986. Relative resistance of potato tubers produced from stem cuttings and seed-piece-propagated plants to *Streptomyces scabies*. Plant Dis. 70:1146-1148.
23. Manzer, F. E., McIntyre, G. A., and Merriman, D. C. 1977. A new potato scab problem in Maine. Maine, Life Sci. Agric. Exp. Stn. Bull. 85:1-24.
24. McQueen, D. A. R., Anderson, N. A., and Schottel, J. L. 1985. Inhibitory reactions between natural isolates of *Streptomyces*. J. Gen. Microbiol. 131:1149-1155.
25. Menzies, J. D. 1959. Occurrence and transfer of a biological factor in soil that suppresses potato scab. Phytopathology 49:648-652.
26. Millard, W. A., and Burr, S. 1926. A study of twenty-four strains of *Actinomycetes* and their relation to types of common scab of potato. Ann. Appl. Biol. 13:580-644.
27. Parmeter, J. R., Jr., Sherwood, R. T., and Platt, W. D. 1969. Anastomosis grouping among isolates of *Thanatephorus cucumeris*. Phytopathology 59:1270-1278.
28. Polsinelli, M., and Beretta, M. 1966. Genetic recombination in crosses between *Streptomyces aureofaciens* and *Streptomyces rimosus*. J. Bacteriol. 91:63-68.
29. Schneider, R. W., ed. 1982. Suppresive Soils and Plant Disease. American Phytopathological Society, St. Paul, MN.
30. Shirling, E. B., and Gottlieb, D. 1969. Cooperative description of type cultures of *Streptomyces*. IV. Species descriptions from the second, third, and fourth studies. Int. J. Syst. Bacteriol. 19:391-512.
31. Thaxter, R. 1891. The potato "scab". Conn. Agr. Exp. Stn. Storrs, Res. Rep. 1890:81-95.
32. Vidaver, A. K. 1976. Prospects for control of phytopathogenic bacteria by bacteriophages and bacteriocins. Annu. Rev. Phytopathol. 14:451-465.
33. Vidaver, A. K., Mathys, M. L., Thomas, M. E., and Schuster, M. L. 1972. Bacteriocins of the phytopathogens *Pseudomonas syringae, P. glycinea,* and *P. phaseolicola*. Can. J. Microbiol. 18:705-713.
34. Waksman, S. A. 1961. The *Actinomycetes*. Vol. 2. The Williams and Wilkins Co., Baltimore, MD.
35. Williams, S. T., Goodfellow, M., Alderson, G., Wellington, E. M. H., Sneath, P. H. A., and Sackin, M. J. 1983. Numerical classification of *Streptomyces* and related genera. J. Gen. Microbiol. 129:1743-1813.