

Streptomyces acidiscabies sp. nov.†

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A new bacterial species is described, for which we propose the name *Streptomyces acidiscabies*. This organism causes a scab disease of potatoes (*Solanum tuberosum*) which may occur in soils with pH values below 5.2. The acid scab symptoms caused by this organism are indistinguishable from the symptoms of common scab caused by *Streptomyces scabies*. In culture, *S. acidiscabies* is distinct from *S. scabies*, having flexuous spore chains, a growth medium-dependent spore mass color ranging from white to orangish red, and a red or yellow, pH-sensitive diffusible pigment rather than melanin. *S. acidiscabies* grows on agar media at pH 4.0 (versus pH 5.0 for *S. scabies*), does not use raffinose as a carbon source, and tolerates higher concentrations of crystal violet (0.5 µg/ml), thallous acetate (10 µg/ml), streptomycin (20 µg/ml), oleandomycin (25 µg/ml), and penicillin G (10 IU/ml) than *S. scabies*. The type strain of *S. acidiscabies* is strain RL-110 (= ATCC 49003).

Potato scab diseases are caused by several *Streptomyces* spp., among which *Streptomyces scabies* predominates. This species is revived and redefined in the accompanying paper (8). Scab diseases are characterized by corky tuber lesions, which may be superficial or pitted. Common scab is generally controlled by maintenance of a soil pH below 5.2. An indistinguishable form of the disease (acid or uncommon scab), which occurs in soils with pH values as low as 4.5, was first detected in Maine in 1953. Early outbreaks of this disease were often associated with a particular Midwestern seed lot of the very susceptible variety 'Chippewa', suggesting that the pathogen was not native to Maine. However, there were also instances of the disease on farms which used no outside seed sources. Discontinuation of harsh types of seed treatment in the early 1950s may have led to the build-up of indigenous pathogens to perceptible levels in such cases, as the acid-tolerant species persists primarily on infected tubers rather than in soil (10). Strains of *Streptomyces* species causing this form of the disease were isolated and described by Bonde and McIntyre (2) and by Manzer et al. (10), who found differences between typical and acid scab strains in pigment, raffinose usage, spore chain morphology, and tolerance of low soil pH values. The work of these authors and the protein electrophoresis characterizations of Hughes et al. (4) demonstrated that the organisms causing acid scab are distinct from those causing common scab in phenotype and ecology. Acid scab is now present in various areas of the Northeast (R. Loria, B. A. Kempter, and A. A. Jamieson, *Am. Potato J.* **63**:440, 1986) and upper Midwest. In this paper, we provide additional descriptions of acid scab strains and propose for them the name *Streptomyces acidiscabies*.

MATERIALS AND METHODS

Strains. The cultures of *Streptomyces* species investigated included the following four acid scab strains recently isolated by Loria et al. (*Am. Potato J.* **63**:440, 1986): strains RL-104 (from New York), RL-45, RL-110^T (= ATCC 49003^T) (T = type strain), and RL-182 (from Maine). The criteria used to select these strains from a much larger isolate collection were inability to use raffinose, flexuous spore

chains, and tolerance of an agar medium having a pH of 4.0. The pathogenicity of these strains was verified on tubers produced from stem cuttings (9). A fifth acid-tolerant strain, strain U30 (= ATCC 49004), was also included; this strain was isolated in Maine in the 1960s and was used as a standard in previous studies (10). The five acid-tolerant strains were compared with the following five typical *S. scabies* strains (pathogenic, melanin-producing, raffinose-using organisms with spiral spore chains) that were isolated and characterized at the same time: strains RL-39 and RL-42 from New York, strains RL-70 and RL-174 from Maine, and strain RL-151 from New Brunswick, Canada. The following three additional strains were included: *Streptomyces tendae* ATCC 19812^T, which was considered by Hutter (5) to be similar to certain common scab strains; strain ATCC 10246, a pathogen that has been transferred to *Streptomyces griseus* (3); and the erroneous (7) "*S. scabies*" neotype strain IMRU 3018 (= ISP 5078) (15). The last two strains were included to represent common scab strains not covered by the original species description. (Quotation marks indicate that a strain does not belong in the revived species *S. scabies* [8].)

Strain characterization. International *Streptomyces* Project methods (13) were used to determine spore surface morphology, spore chain morphology, melanin and soluble pigment formation, and carbohydrate usage. The predominant spore mass pigment was described with a single color and with an additional modifying color in some instances. To determine whether they could be used as carbon sources, simple sugars and polysaccharides were filter sterilized and added to basal agar medium at concentrations of 1.0% (wt/vol). Basal medium contained (per liter) 2.64 g of (NH₄)₂SO₄, 2.38 g of KH₂PO₄, 5.65 g of K₂H₂PO₄ · 3H₂O, 1.00 g of MgSO₄ · 7H₂O, 6.4 mg of CuSO₄ · 5H₂O, 1.1 mg of FeSO₄ · 7H₂O, 7.9 mg of MnCl₂ · H₂O, 1.5 g of ZnSO₄ · 7H₂O, and 15.0 g of agar; the pH was 6.9 (12). The toxicities of inhibitory compounds were determined on modified Bennett agar containing (per liter) 1 g of beef extract, 1 g of yeast extract, 2 g of tryptone, 10 g of glycerol, and 15 g of agar. The toxic compounds tested were selected from those used by Williams et al. (20) and included penicillin G (10 IU/ml), streptomycin sulfate (20 µg/ml), oleandomycin (25 µg/ml), thallous acetate (10 µg/ml), sodium chloride (7%, wt/vol), and crystal violet (0.5 µg/ml). Additional concentrations of these chemicals or of other toxic compounds were screened, but our results are not reported below. The

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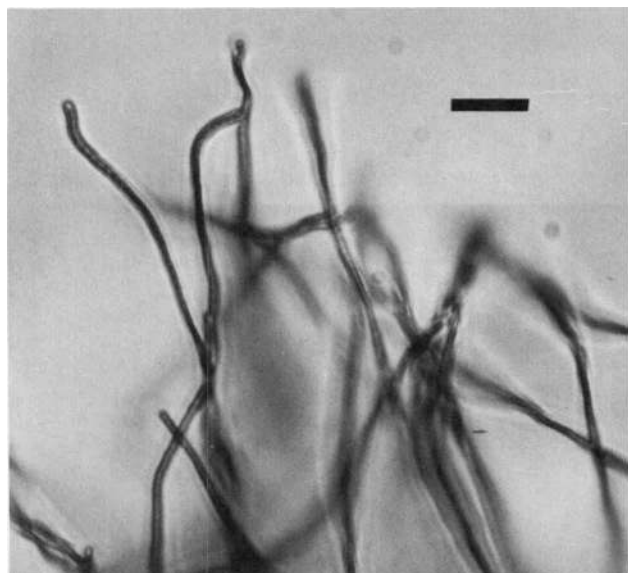


FIG. 1. Sporulating aerial mycelium of strain ATCC 49003^T (= RL-110^T): flexuous spore chains of an organism grown on yeast malt extract agar at 27°C for 14 days. Bar = 10 μ m.

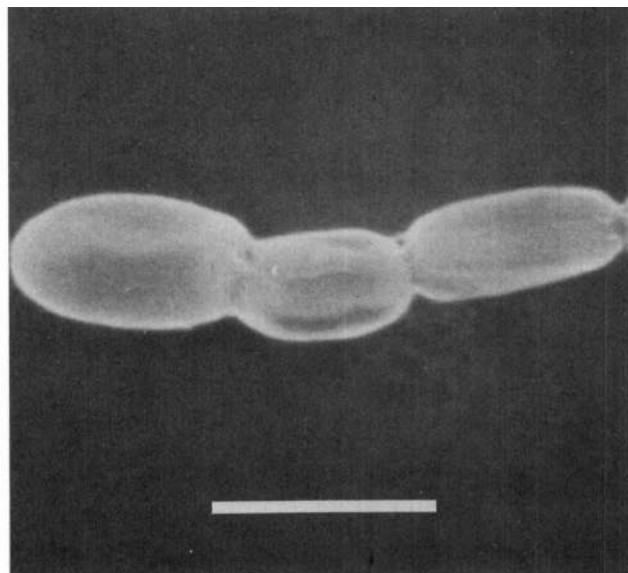


FIG. 2. Smooth spores of strain ATCC 49003^T (= RL-110^T) grown on inorganic salts starch agar at 27°C for 14 days. Scanning electron micrograph. Bar = 1 μ m.

minimum pH that allowed growth was determined on media containing 10.0 g of dextrose per liter, 0.5 g of L-asparagine per liter, 15.0 g of agar per liter, and 40 mM phosphate as the monopotassium, dipotassium, and disodium salts to adjust the pH in 0.5-U intervals from 3.5 to 8.5 (19). For inoculation of agar plates, spores were scraped from salts starch medium plates, suspended in 0.001% Triton X-100, filtered through sterile glass wool, washed, suspended in water, and spread in a single line by using a micropipette. Growth was evaluated after 14 days.

Wall composition (DL- and LL-diaminopimelic acid isomers) was determined by the method of Becker et al. (1). A diffusible pigment was produced by *S. acidiscabies* in modified salts starch agar or broth containing (per liter) 10.0 g of soluble starch (or glycerol), 1.0 g of NaNO₃, 1.0 g of MgCO₃, 0.3 g of K₂PO₄, and 0.5 g of NaCl, with or without 15.0 g of agar per liter (7). This pigment was concentrated from broth by a series of extractions in which we used *n*-butanol with alternating acidic and alkaline aqueous phases. The pK_a of the pigment was determined photometrically in 0.05 M phosphate buffer titrated with HCl or NaOH by measuring the absorbance at 420 nm.

The phenotypes of the acid scab strains were compared with three taxonomic keys derived from the International *Streptomyces* Project (6, 11, 18). Additional comparisons were made with the species descriptions of Shirling and Gottlieb (14–17) and Williams et al. (20).

RESULTS

The five *S. acidiscabies* strains are distinct from typical *S. scabies* isolates, from two atypical scab strains (strains IMRU 3018 and ATCC 10246), from *S. tendae* ATCC 19812^T, and from descriptions of other previously recognized species.

Morphological characteristics. On all standard agar media, the aerial mycelium of *S. acidiscabies* cultures was sympodially branched and formed flexuous spore chains (Fig. 1) which rarely curled at the tip. Fragmentation of the substrate mycelium, sporangium or sclerotium formation, and flagellated spores were not observed. The spores were smooth

and cylindrical, 0.4 to 0.5 μ m in diameter by 0.9 to 1.1 μ m long (Fig. 2); occasionally they were as short as 0.6 μ m. The cell walls contained the LL-diaminopimelic acid isomer.

Pigments. *S. acidiscabies* strains did not produce melanoid pigments on tyrosine or peptone iron agar media (Table 1). When they were mass inoculated onto modified Kutzner inorganic salts glycerol agar, all five strains produced a dark red diffusible pigment which turned yellow when it was acidified with 1 N HCl and then red again when it was treated with 1 N NaOH. Maximum pigment production occurred with 1% (wt/vol) glycerol rather than starch. The pK_a of the extracted pigment was 8.3. Depending on the medium, spore masses were white to salmon pink (Table 1). If concentrated by centrifugation, spores from pink colonies appeared to be dark red. *S. tendae* produced a dissimilar yellow pigment on various media. On the glucose asparagine medium used to determine responses to different pH values, this pigment was bright yellow and diffused at ca. pH 6.5 and above, but was more orange and immobile below this pH.

Physiological characteristics. The responses of *S. acidiscabies* and *S. scabies* strains were uniform within their respective groups. On Pridham-Gottlieb basal medium, *S. acidiscabies* used L-arabinose, D-fructose, D-glucose, i-inositol, D-mannitol, rhamnose, sucrose, and D-xylose but not raffinose as sole carbon sources (Table 1). The responses of the strains to toxic compounds also had differential value. In summary, *S. acidiscabies* cultures were distinct from all other cultures in spore color, diffusible pigment production, acid tolerance, and at least four additional criteria (Table 1). The five *S. acidiscabies* strains were further characterized in order to compare them with a numerical taxonomy scheme of the genus *Streptomyces* (20). All of the strains had the ability to degrade arbutin, polygalacturonate (at pH 7.3), starch, xanthine, and xylan. They also had the ability to use as sole nitrogen sources L-hydroxyproline, L-histidine, L-methionine, and L-valine. Only strains RL-45, RL-104, and RL-110^T were able to use L-phenylalanine. Only strain RL-45 was able to use salicin as a sole carbon source. Growth did not occur in agar media containing 10 μ g of sodium azide per ml, 1 μ g of crystal violet per ml, or 7%

TABLE 1. Characteristics of *S. acidiscabies* and other species

Characteristic	<i>S. acidiscabies</i> isolates	<i>S. scabies</i> isolates	<i>S. tendae</i> ATCC 19812 ^T	" <i>S. scabies</i> " IMRU 3018	<i>S. griseus</i> ATCC 10246
Spore mass color on ^a :					
Salts starch medium	W(O,Gy)	Gy	Gy	Gy	Gy
Oatmeal medium	W(O)	Gy(Y)	Gy	Gy	Gy
Glycerol asparagine medium	W(O,Gy)	Gy(Y,B)	Gy	Gy	Gy
Yeast malt medium	W(O,Gy)	Gy(Br)	Gy	Gy	(Y)W
Reverse mycelium color on ^a :					
Salts starch medium	Br,Gy(Y)	Gy(Y)	Y	Gy	(Br)W
Oatmeal medium	Br(Y)	Gy(Y,Br)	Gy	(B)Gy	(Y)W
Glycerol asparagine medium	Br(Y)	Gy(Y)	Y	(B)Gy	(Br)W
Yeast malt medium	Br(Y)	Br(Y,Gy)	Y	Gy	(Y)W
Spore chain morphology	Flexuous	Spiral	Flexuous	Flexuous	Flexuous
Spore ornamentation	Smooth	Smooth	Smooth	Smooth ^b	NA ^c
Melanin from tyrosine	—	+	—	—	—
Pigment on peptone iron medium	—	+	—	—	—
Diffusible pigment ^a	R/Y ^d	—	Y/O	—	—
DAP optical isomer ^e	LL	LL	LL	LL	LL
Carbon source usage					
L-Arabinose (1%, wt/vol)	+	+	+	+	+
D-Fructose (1%, wt/vol)	+	+	+	+	+
D-Glucose (1%, wt/vol)	+	+	+	+	+
D-Mannitol (1%, wt/vol)	+	+	+	+	+
Raffinose (1%, wt/vol)	—	+	—	—	—
Rhamnose (1%, wt/vol)	+	+	+	+	—
Sucrose (1%, wt/vol)	+	+	—	—	+
D-Xylose (1%, wt/vol)	+	+	+	+	—
meso-Inositol (1%, wt/vol)	+	+	+	—	—
Minimum pH for growth	4.0	5.0	4.5	5.5	5.5
Growth in the presence of:					
Thallium acetate (100 µg/ml)	—	—	—	—	+
Thallium acetate (10 µg/ml)	+	—	+	+	+
Sodium chloride (7%, wt/vol)	—	—	+	+	+
Crystal violet (0.5 µg/ml)	+	—	+	+	—
Phenol (0.1%, wt/vol)	+	—	+	+	+
Penicillin G (10 IU/ml)	+	—	+	+	+
Oleandomycin (100 µg/ml)	—	—	+	—	+
Oleandomycin (25 µg/ml)	+	—	+	+	+
Streptomycin (20 µg/ml)	+	—	—	—	+

^a B, Blue; Br, brown; Bk, black; Gy, grey; O, orange; R, red; W, white; Y, yellow. Colors in parentheses indicate secondary hue(s) of predominant colors.

^b Data from reference 13.

^c NA, Not available. Strain ATCC 10246 spores are presumed to be smooth.

^d Color on alkaline media/color on acid media.

^e DAP, Diaminopimelic acid.

NaCl, but did occur in media containing 100 µg of potassium tellurite per ml. The methods used for these tests are described elsewhere (8, 20).

DISCUSSION

The five *S. acidiscabies* strains which we studied were nearly identical in their morphological and physiological characteristics. They differed perceptibly only in the degree of pigment production and the ability to grow on media containing salicin or phenylalanine as a sole carbon or nitrogen source. This uniformity may indicate that many acid scab strains in the northeastern United States are derived from a common source. Melanin is not produced by acid scab strains, which is further evidence that tyrosinase is not essential in the disease process. *S. acidiscabies* can grow on agar media at a lower pH than other scab-causing isolates (Table 1). Optimum growth, as evidenced by mycelium density and rate of colony development, occurred at pH 7 and above, indicating that *S. acidiscabies* is acid tolerant rather than acidophilic. This is consistent with the ability of this species to cause disease of reduced severity in soils that

are too acidic for *S. scabies* (2). The distinct differences between *S. scabies* and *S. acidiscabies* suggest that these two species are not closely related and that their ability to infect plants developed independently. When the percentages of traits held in common between *S. acidiscabies* and other scab pathogens or reference strains were calculated (8), little similarity was found. *S. acidiscabies* strains were most similar to *Streptomyces setonii* ATCC 25497^T (a weak pathogen) and *S. tendae* ATCC 19812^T (level of similarity, 80% in each case). The level of similarity to *S. scabies* was only 64%. When *S. acidiscabies* was compared with the major *Streptomyces* groups of Williams et al. (20), no strong similarities were identified. *S. acidiscabies* was most similar to the following cluster groups: *Streptomyces griseoruber* (67%), *Streptomyces fulvissimus* (65%), *Streptomyces aureofasciens* (65%), *Streptomyces albidoflavus* (64%), and *Streptomyces exfoliatus* (63%).

***Streptomyces acidiscabies* sp. nov.** *Streptomyces acidiscabies* (ac.i.di.sca'bies. L. adj. *acidus*, acid; L. n. *scabies*, mange; acidiscabies, referring to the ability of the organism to cause acid scab of potatoes). Spores are 0.4 to 0.5 by 0.6 or 0.9 to 1.1 µm, smooth, and white (reddish on certain

high-pH media) and are borne in mature flexuous chains containing 20 or more spores. A diffusible pigment is produced which is red above pH 8.3 and golden yellow below pH 8.3. Melanin is not produced. L-Arabinose, D-fructose, D-glucose, D-mannitol, rhamnose, sucrose, and D-xylose are used as carbon sources, but not raffinose is not. The guanine-plus-cytosine content of this species is 71 mol%. *S. acidiscabies* differs from all other streptomycetes that cause typical raised or pitted symptoms and have white spores, red pigment, and acid tolerance. It differs in at least one major characteristic from all other strains described in the International *Streptomyces* Project having red pigments or spores. Species characteristics, including those which differentiate *S. acidiscabies* from other pathogens, are shown in Table 1. *S. acidiscabies* is placed in the genus *Streptomyces*, as it possesses typical morphology and cell walls with the LL-diaminopimelic acid isomer. To date, *S. acidiscabies* has been isolated only from potatoes (*Solanum tuberosum*), and its pathogenicity on other species has not been determined. The ability of this species to persist in soil is relatively poor, and it is usually transmitted by infected seed tubers (10). The five strains which we studied (strains RL-45, RL-104, RL-110^T [= ATCC 49003^T], RL-182, and U30 [= ATCC 49004]) are presently the only examples of the species in culture. Specific characteristics of type strain RL-110 (= ATCC 49003) are listed in Table 1 or are mentioned above. The phenotypes of other strains studied previously by Bonde and McIntyre (2) are in agreement with the phenotype described here. All strains appear to differ very little from each other.

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LITERATURE CITED

1. Becker, B., M. P. Lechevalier, R. E. Gordon, and H. A. Lechevalier. 1964. Rapid differentiation between *Nocardia* and *Streptomyces* by paper chromatography of whole-cell hydrolysates. *Appl. Microbiol.* **12**:421-423.
2. Bonde, M. R., and G. A. McIntyre. 1968. Isolation and biology of a *Streptomyces* sp. causing potato scab in soils below pH 5.0. *Am. Potato J.* **45**:273-278.
3. Gordon, R. E., and C. Horan. 1968. A piecemeal description of *Streptomyces griseus* (Krausky) Waksman and Henrici. *J. Gen. Microbiol.* **50**:223-233.
4. Hughes, C., R. C. McCrum, and F. E. Manzer. 1971. Protein and esterase electrophoretic patterns of *Streptomyces* spp. isolated from Maine potatoes. *Am. Potato J.* **48**:206-213.
5. Hütter, R. 1967. *Systematic der Streptomyceten*. Karger, Basel.
6. Küster, E. 1972. Simple working key for the classification and identification of named taxa included in the International *Streptomyces* Project. *Int. J. Syst. Bacteriol.* **22**:139-148.
7. Kutzner, H. J. 1979. The family Streptomycetaceae, p. 2028-2090. In M. P. Starr, H. Stolp, H. G. Trüper, A. Balows, and H. G. Schlegel (ed.), *The prokaryotes*. Springer-Verlag, Berlin.
8. Lambert, D. H., and R. Loria. 1989. *Streptomyces scabies* sp. nov., nom. rev. *Int. J. Syst. Bacteriol.* **39**:387-392.
9. Loria, R., and B. A. Kempter. 1986. Relative resistance of potato tubers produced from stem cuttings and seed-piece-propagated plants to *Streptomyces scabies*. *Plant Dis.* **70**:1146-1148.
10. Manzer, F. E., G. A. McIntyre, and D. C. Merriam. 1977. A new potato scab problem in Maine. *Maine Life Sci. Agric. Exp. Stn. Bull.* **85**:1-24.
11. Nonomura, H. 1974. Key for classification and identification of 458 species of the streptomycetes included in the ISP. *J. Ferment. Technol.* **52**:78-92.
12. Pridham, T. G., and D. Gottlieb. 1948. The utilization of carbon compounds by some Actinomycetales as an aid for species determination. *J. Bacteriol.* **56**:107-114.
13. Shirling, E. B., and D. Gottlieb. 1966. Methods for characterization of *Streptomyces* species. *Int. J. Syst. Bacteriol.* **16**:313-340.
14. Shirling, E. B., and D. Gottlieb. 1968. Cooperative description of type cultures of *Streptomyces*. II. Species descriptions from first study. *Int. J. Syst. Bacteriol.* **18**:69-189.
15. Shirling, E. B., and D. Gottlieb. 1968. Cooperative description of type cultures of *Streptomyces*. III. Additional species descriptions from first and second studies. *Int. J. Syst. Bacteriol.* **18**:279-391.
16. Shirling, E. B., and D. Gottlieb. 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.
17. Shirling, E. B., and D. Gottlieb. 1972. Cooperative description of type strains of *Streptomyces*. V. Additional descriptions. *Int. J. Syst. Bacteriol.* **22**:265-394.
18. Szabo, I. M., M. Marton, I. Buti, and C. Fernandez. 1975. A diagnostic key for the identification of "species" of *Streptomyces* and *Streptoverticillium* included in the International *Streptomyces* Project. *Acta Bot. Acad. Sci. Hung.* **21**:387-418.
19. Williams, S. T., F. L. Davies, C. I. Mayfield, and M. R. Khan. 1971. Studies on the ecology of actinomycetes in soil. II. The pH requirements of streptomycetes from two acid soils. *Soil Biol. Biochem.* **3**:187-195.
20. Williams, S. T., M. Goodfellow, G. Alderson, E. M. H. Wellington, P. H. A. Sneath, and M. J. Sackin. 1983. Numerical classification of *Streptomyces* and related genera. *J. Gen. Microbiol.* **129**:1743-1813.