Techniques

Fatty Acid Analysis of Pathogenic and Suppressive Strains of *Streptomyces* Species Isolated in Minnesota

T. C. R. Ndowora, L. L. Kinkel, R. K. Jones, and N. A. Anderson

Department of Plant Pathology, University of Minnesota, St. Paul 55108.

Published as paper 21,942 of the contribution series of the Minnesota Agricultural Experiment Station based on research conducted under Project 22-18H, supported by a grant from the University of Minnesota College of Agriculture Parker Sanders Fund and the United Methodist Church.

Accepted for publication 22 October 1995.

ABSTRACT

Ndowora, T. C. R., Kinkel, L. L., Jones, R. K., and Anderson, N. A. 1996. Fatty acid analysis of pathogenic and suppressive strains of *Streptomyces* species isolated in Minnesota. Phytopathology 86:138-143.

Cellular fatty acid analysis was assessed for identification and differentiation of seven suppressive strains of *Streptomyces* spp., 19 pathogenic strains of *Streptomyces* spp., and a *S. albidoflavus*-type strain (ATCC 25422). An average of 16 to 18 fatty acids in varying quantities were identified in each of the strains. The pathogenic *S. scabies* strains and the *S. albidoflavus*-type strain were distinguishable from *S. acidiscabies* based on average linkage cluster analysis of cellular fatty acids. With the exception of *S. acidiscabies*, pathogenic *Streptomyces* strains were distinguished from suppressive strains. To assess the utility of fatty acid composition for classification of *Streptomyces* strains, grouping of strains based on cellular fatty acid composition was compared to groups based on inhibitory reactions, antibiotic production, and sensitivity to other antibiotics and was similar but not identical.

Additional keywords: biological control, potato scab, rhizosphere.

Potato scab, an important disease that affects developing tubers, is primarily caused by *S. scabies* (21) and *S. acidiscabies* (3,22, 29). Several other *Streptomyces* spp. also cause potato scab (2,7), including some with characteristics similar to those of the *S. albidoflavus* group of Williams et al. (13,21,35). In some cases, pathogenic *Streptomyces* strains cannot be identified (9). Identification of pathogenic *Streptomyces* species isolated from scab lesions on potatoes is difficult partly as a consequence of the confusing state of *Streptomyces* taxonomy.

Scab reduces the economic value of potatoes by producing superficial corklike layers (russet scab) or causing tissues to be erumpent or cushion-like (raised scab). Diseased tissue also may extend into the tuber, resulting in shallow or deep pit scab (14). Potato scab can be controlled by crop rotation, cultivar resistance, soil fumigation, soil acidification with sulfur compounds (14), and irrigation (1). In Minnesota, scab is controlled by crop rotation and the use of resistant potato cultivars. Market factors, including chip color and response to other diseases, such as early dying and late blight, have resulted in an increase in plantings of scab-susceptible cultivars in Minnesota. Biological control, such as the use of suppressive soils (30) or antibiotic-producing *Streptomyces* strains antagonistic to potato scab pathogens (12, 25,26,27), offers an alternative, environmentally acceptable method for control of potato scab.

In Minnesota, a breeding plot used for the selection of scab-resistant plant lines was maintained under continuous potato culture beginning in 1943. In 1965 susceptible lines planted in this plot began to show a decline in scab infection, and the plot was abandoned in 1971 (27). In 1987 *Streptomyces* strains from potato tubers grown in this plot demonstrated antibiotic activity and the ability to suppress growth of pathogenic *S. scabies* in vitro (27). The addition of soil from the suppressive scab-breeding plot to a scab-conducive plot significantly reduced disease (27). Since that time, suppressive strains of diverse morphology have been isolated from potato tubers grown in the suppressive soil, and their antibiotic activities against pathogens have been determined (24,25). In experiments carried out from 1988 to 1992, two antibiotic-producing suppressive strains (PonR [*S. scabies*] and PonSSII [*S. diastatochromogenes*]) suppressed pathogenic *S. scabies* in vitro and significantly reduced disease in the field (26). These suppressive strains may be useful as biological control agents against many potato scab pathogens.

Additional research is required to maximize disease control using suppressive *Streptomyces* strains. Ecological studies on rhizosphere interactions of pathogenic and suppressive strains of *Streptomyces* require a means of distinguishing between these groups. *Streptomyces* taxonomy involves the analysis of several morphological characteristics: color of aerial mycelium, substrate mycelium, and soluble pigments; physiological characterization, including melanin production and carbon source utilization; spore wall ornamentation; and ability to produce antibiotics (32). This process is laborious, time-consuming, and prone to error because of the subjectiveness and variability of some of the tests. Additionally, this method is not practical for use in ecological studies because of the large numbers of strains that require identification.

Many methods have been used for rapid identification and differentiation of bacteria inoculated into the rhizosphere (15,16,19). One of the most promising methods for rapid identification of unknown bacterial isolates relies on analysis of qualitative and quantitative differences in cellular fatty acids (4,6,10). The objective of this work was to determine whether cellular fatty acid composition could be used to identify and differentiate pathogenic *S. scabies* and *S. acidiscabies* strains and a *S. albidoflavus*-type strain from antibiotic-producing suppressive strains. Also, cellular fatty acid composition was analyzed for a subset of pathogenic and suppressive *Streptomyces* strains previously grouped based on biological criteria (28) to determine whether groups based on fatty acid composition were similar.

Corresponding author: L. L. Kinkel; E-mail address: lindak@puccini.crl.umn.edu

Publication no. P-1995-1220-01R

© 1996 The American Phytopathological Society

MATERIALS AND METHODS

Bacterial strains. Sixteen pathogenic *S. scabies* strains, including the *S. scabies*-type strain ATCC 49173, and seven suppressive strains that produced antibiotics in vitro and suppressed growth of pathogenic *S. scabies* strains in pot and field experiments (23) were studied (Table 1). The suppressive *S. scabies* strain, PonR, is also weakly virulent (28). Plant pathogenic *S. acidiscabies* strains RL 104 and RL 182 (22), a russet scab strain, and the *S. albidoflavus*-type strain (ATCC 25422) also were analyzed. Isolates were stored in 20% glycerol at –10°C.

Cellular fatty acid analysis. Cellular fatty acid analysis was conducted using the Microbial Identification System (MIS, version 3.2) developed by Microbial ID (MIDI, Newark, DE). *Streptomyces* strains were grown on nonamended oatmeal agar (20) for routine culturing. For each of the 27 *Streptomyces* strains, 20 single-spore colonies were evaluated for cellular fatty acid composition. Individual colonies were inoculated separately into 20 ml of Trypticase soy broth (Becton Dickinson Microbiology System, Cockeysville, MD) in 125-ml Erlenmeyer flasks containing 5 g of glass beads. Flasks were shaken at 225 cycles per min at 28°C for 72 h, and cells were harvested after centrifugation at 2,300 × *g* for 10 min at 4°C. Cellular fatty acids were extracted from a 100- to 110-mg wet weight sample from each flask according to the method of Sasser (33). Fatty acid methyl esters from each strain were separated using a Hewlett-Packard (Palo Alto, CA) gas chromatograph model 5890 fitted with a fused silica capillary column (25 m × 0.2 mm internal diameter) cross-linked with 5% phenyl methyl silicone and equipped with a flame ionization detector. Fatty acid methyl ester peaks eluting from the column were detected and identified based on retention times (11) and were expressed as a percentage of total fatty acids in the sample.

Data evaluation. The library generation software included in MIS was used to develop a library for the 27 *Streptomyces* strains. Each strain comprised a library entry. Each library entry was a profile of the mean percentages and standard deviations (SD) of fatty acids from 20 replicate samples of each *Streptomyces* strain. The fatty acids used to create a library entry were those that were reproducibly detected in the 20 replicates. Fatty acids were not used to create the library entry when the product of the mean percent composition multiplied by the percentage of total replicates that contained the fatty acid was less than a quality threshold of 0.25.

Variability in fatty acid composition among replicates of a strain. Variability among replicates of a single strain was assessed with respect to individual fatty acids and overall fatty acid composition. Variability in individual fatty acids was determined by calculating the variance-to-mean ratio of each fatty acid in a strain, and variability in overall fatty acid composition among replicates of a strain was determined by measuring the distance of each replicate from the library mean of each strain using software included in MIS. The distance from the library mean in 3-D space gives the similarity index, which can range from 0.0 to 1.0. A similarity index of 0.5 is 3 SD from the library mean (MIS Operating Manual, version 3.0). Additionally, principal component analysis (PCA; Statistical Analysis System [SAS], release 6.08, SAS Institute, Cary, NC) of replicate samples of a subset of the *Streptomyces* strains was used to determine the amount of variability among replicates.

Variability in fatty acid composition among strains. Variability among all the pathogenic and suppressive *Streptomyces* strains based on cellular fatty acid composition was assessed with the average linkage cluster analysis procedure using software included in the MIS.

RESULTS

Twenty-six fatty acids were detected in the 27 *Streptomyces* strains, with thirteen fatty acids common to all strains. Each *Streptomyces* strain had a total of between 16 and 18 fatty acids. The fatty acids 15:0 *anteiso* and 16:0 *iso* each made up at least 10% of whole cell fatty acid composition of all strains (Tables 2 and 3). Analysis of variance (ANOVA) of individual fatty acids showed significant differences in the level of some fatty acids in the pathogens compared to suppressive strains. Suppressive strains had significantly greater quantities of the fatty acids 15:0 *anteiso*, 17:0 *anteiso*, and 17:1 *anteiso* C and significantly smaller quantities of the fatty acids 14:0 *iso*, 16:0 *iso*, and 16:1 *iso* H compared to the pathogenic strains (ANOVA, $P \leq 0.05$).

In general, the most abundant fatty acid in a strain differed between the pathogenic and suppressive *Streptomyces* strains. The most abundant fatty acid in the suppressive strains was 15:0 *anteiso*, which averaged 25.78% of whole-cell fatty acid composition compared to 14.33% in the pathogenic strains. The most abundant fatty acid in the pathogens (including *S. acidiscabies* RL 104) was 16:0 *iso*, which averaged 28.52% compared to 16.07% in the suppressive strains. An exception to this was the pathogen *S. acidiscabies* (RL 182) in which the major fatty acid was 15:0 *anteiso* (25.71%). A second exception was the suppressive strain PonR (taxonomically a *S. scabies*) in which 16:0 *iso* (25.29%) was the major fatty acid, the same fatty acid found in the pathogens.

Variability in fatty acid composition among replicates of a strain. Variability among replicates of a strain was consistently low. The variance-to-mean ratio of the six most abundant fatty acids in each strain ranged from 0.04 to 0.20. The variance-to-mean ratio of each of all fatty acids in each strain was less than

TABLE 1. *Streptomyces* strains analyzed for cellular fatty acids

Strain	Species[x]	Source[y]
Pathogenic strain		
ATCC 49173	*S. scabies*	New York
BC	*S. scabies*	Becker, MN
BEET	*S. scabies*	Becker, MN
FLII	*S. scabies*	Florida
NC	*S. scabies*	Becker, MN
PONC	*S. scabies*	Becker, MN
PONP	*S. scabies*	Becker, MN
RB2	*S. scabies*	Becker, MN
RB3	*S. scabies*	Becker, MN
RB3II	*S. scabies*	Becker, MN
RB4	*S. scabies*	Becker, MN
RB5	*S. scabies*	Becker, MN
ROY	*S. scabies*	Royalton, MN
87	*S. scabies*	Grand Rapids, MN
88	*S. scabies*	Grand Rapids, MN
89	*S. scabies*	Grand Rapids, MN
CRYS "Russet scab"	Unknown	Grand Forks, ND
RL 104	*S. acidiscabies*	New York
RL 182	*S. acidiscabies*	Maine
ATCC 25422	*S. albidoflavus*[z]	
Pathogen suppressive strain		
15	Unknown	Grand Rapids, MN
32	Unknown	Grand Rapids, MN
93	Unknown	Grand Rapids, MN
PonR	*S. scabies*	Becker, MN
PonSSI	*S. diastatochromogenes*	Grand Rapids, MN
PonSSII	*S. diastatochromogenes*	Grand Rapids, MN
PonSSR	*S. albogriseolus*	Grand Rapids, MN

[x] Identification of strains to species was done by Liu (23) using methods defined at the International *Streptomyces* Project of 1966 (34). Determination of species of strains labeled as unknown was not possible using this method.

[y] *Streptomyces* strains from Becker, MN, Grand Forks, ND, and Grand Rapids, MN, were isolated from scab lesions or lenticels of potato tubers grown in fields at the locations indicated. *S. acidiscabies* strains were obtained from R. Loria, and ATCC isolates were purchased from the American Type Culture Collection, Rockville, MD.

[z] The *S. albidoflavus*-type strain (ATCC 25422) is representative of the *S. albidoflavus* group of Williams et al. (13,35).

0.55, with most within the range of 0.04 to 0.25. Similarity indices for replicate samples of a strain compared to their library means ranged from 0.650 to 0.989. Variability in replicate samples of a strain is illustrated for a subset of the strains using PCA (Fig. 1). Analysis of fatty acids of pathogenic strains by PCA also showed low variability among replicates of a strain (data not shown).

Variability in fatty acid composition among strains. Cellular fatty acid compositions of the 27 strains were compared by performing average linkage cluster analysis (Fig. 2). Suppressive strains 32 and 93 were indistinguishable, as were suppressive strains PonSSI and PonSSII. In both cases, strains in each pair were related at a euclidean distance of less than 2. Strains PonSSI and

TABLE 2. Library profiles of cellular fatty acids of pathogenic *Streptomyces* strains[v]

Fatty acid[w]	*S. scabies* ($n = 15$)	*S. scabies* (ATCC 49173)	*S. acidiscabies* (RL 104)	*S. acidiscabies* (RL 182)	*S. albidoflavus* (ATCC 25422)	*Streptomyces* sp. (Crys "russet scab")
12:0 *iso*	–[x]	–	–	–	–	0.4
13:0 *anteiso*	–	–	–	–	–	0.4
14:0 *iso*	11.0 a[y]	10.3 a	6.7 d	7.8 cd	8.7 c	10.0 b
14:0	–	–	0.6 b	0.8 a	–	0.5 b
15:0 *iso*	9.2 a	9.0 a	7.4 b	6.3 c	9.7 a	8.9 a
15:0 *anteiso*	11.0 d	10.4 d	20.6 b	25.7 a	15.6 c	10.2 d
15:1 (B)[z]	0.8 b	1.2 a	0.5 c	0.6 c	–	1.0 a
15:0	5.3 b	7.0 a	4.0 c	6.7 a	4.3 c	6.1 a
16:1 *iso* (H)	7.3 b	7.3 b	4.4 d	2.6 e	6.0 c	12.6 a
16:0 *iso*	27.6 bc	26.0 d	26.4 cd	21.3 e	28.7 b	31.4 a
16:0 *anteiso*	–	–	–	–	0.5	–
16:1 *cis*-9	6.9 b	7.6 a	8.5 a	7.8 a	2.5 d	5.0 c
15:0 *anteiso*-2OH	–	–	–	–	3.9	–
16:0	5.1 c	5.1 c	7.4 b	8.5 a	3.5 d	2.2 e
16:0 9-methyl	3.7 a	3.8 a	1.5 c	1.1 d	2.3 b	2.7 b
17:1 *anteiso* (C)	2.3 a	2.2 a	2.3 a	1.9 b	2.1 ab	2.2 a
17:0 *iso*	2.0 a	1.8 b	1.2 c	0.9 d	1.9 ab	0.7 d
17:0 *anteiso*	4.6 b	3.9 bc	5.7 a	5.8 a	4.9 b	2.7 c
17:1 *cis*-9	1.5 b	2.2 a	0.6 e	0.8 d	1.4 bc	1.3 c
17:0 *cyclo*	0.7 d	1.0 c	1.9 b	1.1 c	2.8 a	–
17:0	0.5 c	0.8 a	–	0.4 c	0.6 b	–
18:1 *iso* (H)	–	–	–	–	0.7	–

[v] *Streptomyces* strains were cultured in Trypticase soy broth for 72 h at 28°C before fatty acid extraction and analysis.
[w] Fatty acids in each strain are expressed as a percentage of whole-cell fatty acid composition. Fatty acid profiles of strains are means of 20 independent replicate analyses. Fatty acids were named based on time taken to pass through the gas chromatograph capillary column (retention time) (11).
[x] – indicates the fatty acid mean multiplied by the percentage of replicates of a strain with fatty acid is below a quality threshold of 0.25.
[y] Within individual rows (=single fatty acid) numbers followed by the same letter are not significantly different (LSD mean separation used, $P < 0.05$).
[z] A fatty acid followed by a letter in parentheses belongs to the family of the particular fatty acid for which the exact location of the double bond is unknown. Each member of the family with a double bond in a different location is designated by a different letter (Microbial ID [MIDI], Newark, DE).

TABLE 3. Library profiles of cellular fatty acids of scab-suppressive *Streptomyces* strains[u]

Fatty acid[v]	*Streptomyces* sp. (15)	*Streptomyces* sp. (32 and 93)	*S. diastatochromogenes* (PonSSI and PonSSII)	*S. scabies* (PonR)	*S. albogriseolus* (PonSSR)
13:0 *iso*	–[w]	–	0.4 a	0.3 b	0.3 b
13:0 *anteiso*	–	–	0.7	–	–
14:0 *iso*	2.4 c[x]	5.3 b	5.5 b	7.6 a	1.4 c
14:0	0.6 b	0.8 a	–	–	–
15:0 *iso*	11.0 c	7.8 d	15.9 a	13.5 b	14.0 b
15:0 *anteiso*	32.8 a	22.5 c	26.2 b	21.5 d	21.7 cd
15:1 (B)[y]	–	–	1.0 a	0.9 a	0.3 b
15:0	1.6 d	4.6 ab	4.9 a	3.9 b	3.0 c
16:1 *iso* (H)	2.4 bc	3.4 a	1.6 c	3.2 ab	2.7 ab
16:0 *iso*	15.2 c	20.8 b	13.3 d	25.3 a	15.0 cd
16:1 *cis*-9	5.6 c	10.9 a	7.9 b	4.2 d	4.2 d
16:0	6.1 b	8.5 a	3.8 c	2.3 d	4.2 c
Unknown ECL[z] = 16.048	–	–	–	0.5	–
16:0 9-methyl	2.8 d	2.1 e	4.9 b	3.4 c	6.4 a
17:1 *anteiso* (C)	4.4 b	2.7 d	2.6 d	3.8 c	5.9 a
17:0 *iso*	1.8 d	1.3 e	3.1 b	2.1 c	5.3 a
17:0 *anteiso*	9.0 b	5.4 d	6.9 c	6.5 c	12.8 a
17:1 *cis*-9	0.5 e	1.7 a	0.8 c	0.7 d	1.4 b
16:0 *iso*-2OH	–	–	–	–	0.4
17:0 *cyclo*	2.5 a	0.3 b	–	–	–
17:0	–	0.6 a	–	–	0.5 a
Unknown ECL = 17.595	0.9	–	–	–	–

[u] *Streptomyces* strains were cultured in Trypticase soy broth for 72 h at 28°C before fatty acid extraction and analysis.
[v] Fatty acids in each strain are expressed as a percentage of whole-cell fatty acid composition. Fatty acid profiles of strains are means of 20 independent replicate analyses. Fatty acids were named based on time taken to pass through the gas chromatograph capillary column (retention time) (11).
[w] – indicates the fatty acid mean multiplied by the percentage of replicates of a strain with fatty acid is below a quality threshold of 0.25.
[x] Within individual rows (=single fatty acid) numbers followed by the same letter are not significantly different (LSD mean separation used, $P < 0.05$).
[y] A fatty acid followed by a letter in parentheses belongs to the family of the particular fatty acid for which the exact location of the double bond is unknown. Each member of the family with a double bond in a different location is designated by a different letter (Microbial ID [MIDI], Newark, DE).
[z] ECL = equivalent carbon length.

PonSSII were related to strain PonSSR at a euclidean distance of 10.55. Strains 15 and PonR were distinct from other suppressive strains examined (Fig. 2).

The 14 pathogenic *S. scabies* strains from Minnesota as well as the Florida *S. scabies* strain and the ATCC 49173-type strain clustered at a euclidean distance of less the 10. Relatedness at a euclidean distance of less than 10 is generally used as a taxonomic standard for distinguishing species (33), suggesting that the pathogenic *S. scabies* strains analyzed all belong to the same species. Pathogenic *S. scabies* strains and the *S. albidoflavus*-type strain were closely related at a euclidean distance of 8.44 and were distinguishable from *S. acidiscabies* RL 104 and RL 182 (Fig. 2).

Suppressive and pathogenic strains were separated into two major clusters related at a euclidean distance of 19.65. The exception to this was *S. acidiscabies*, in which case both pathogenic strains RL 104 and RL 182 grouped with the suppressive strains. Suppressive strain PonR, which is taxonomically considered *S. scabies* (23), clustered with the suppressive strains and not the pathogens. The *S. scabies* cluster was divided further into two minor subclusters. To clarify the validity of the MIS analysis, dendrogram generation using average linkage cluster analysis on fatty acids of all 27 strains was performed using SAS. This analysis produced the same clusters and showed the same relationships among strains as those shown in Figure 2 (data not shown).

Comparison of *Streptomyces* groups based on fatty acid composition and groups based on biological characteristics. Among 17 *Streptomyces* strains examined previously, 7 groups were recognized based on inhibitory reactions (lethal zygosis, antibiotic-like inhibitions, and no antagonism), antibiotic production, and sensitivity to antibiotics (28). The fatty acids of these 17 strains were subjected to PCA and compared to the 7 groups (Fig. 3). Groups of the 17 strains based on fatty acid composition were similar, but not identical, to groups formed based on the other attributes. *Streptomyces* strains Crys, FLII, and PonSSR did not cluster with any strain based on lethal zygosis, antibiotic production, or fatty acid composition. The *S. scabies* strains assigned to group 1 based on the other tests clustered together based on fatty acid composition. The group 2 strains RB3 and RB3II clustered with the group 1 strains.

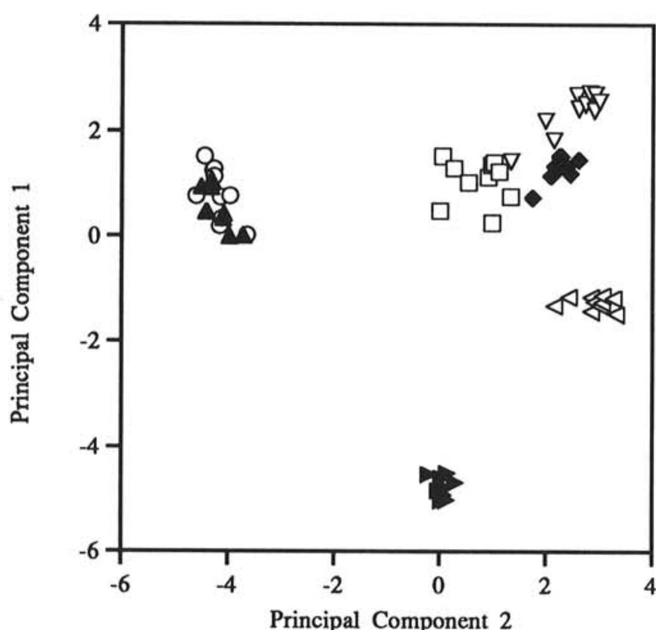

Fig. 1. Variability in whole-cell fatty acid composition among replicates of *Streptomyces* strains, shown for 10 of 20 independent replicate samples of each suppressive strain. The isolates are 15 = ►; 32 = ▲; 93 = ○; PonR = □; PonSSI = ♦; PonSSII = ▽; and PonSSR = ◁.

DISCUSSION

On the basis of cellular fatty acid composition, pathogenic strains of *S. scabies* and the type strain of *S. albidoflavus* were distinguished from pathogen-suppressive strains of *Streptomyces*. This suggests that cellular fatty acid composition may provide a useful means for distinguishing among pathogenic and suppressive *Streptomyces* strains and may provide insight into the biology of *Streptomyces* strains.

Identification of *Streptomyces* strains in ecological studies using traditional taxonomic criteria is labor-intensive and may be complicated by minor physiological differences among strains. Additionally, identification schemes using traditional taxonomic methods may not provide significant insight into strain ecology. For example, in ecological studies of the population dynamics of *S. scabies* in the potato rhizosphere, only 6% of *Streptomyces* strains identified as *S. scabies* based on taxonomic criteria were pathogenic on potato (15). Use of fatty acid analysis to distinguish and identify strains of *Streptomyces* in ecological studies may be more useful than taxonomic criteria because of its relative efficiency and the insight it can provide into strain biology. For

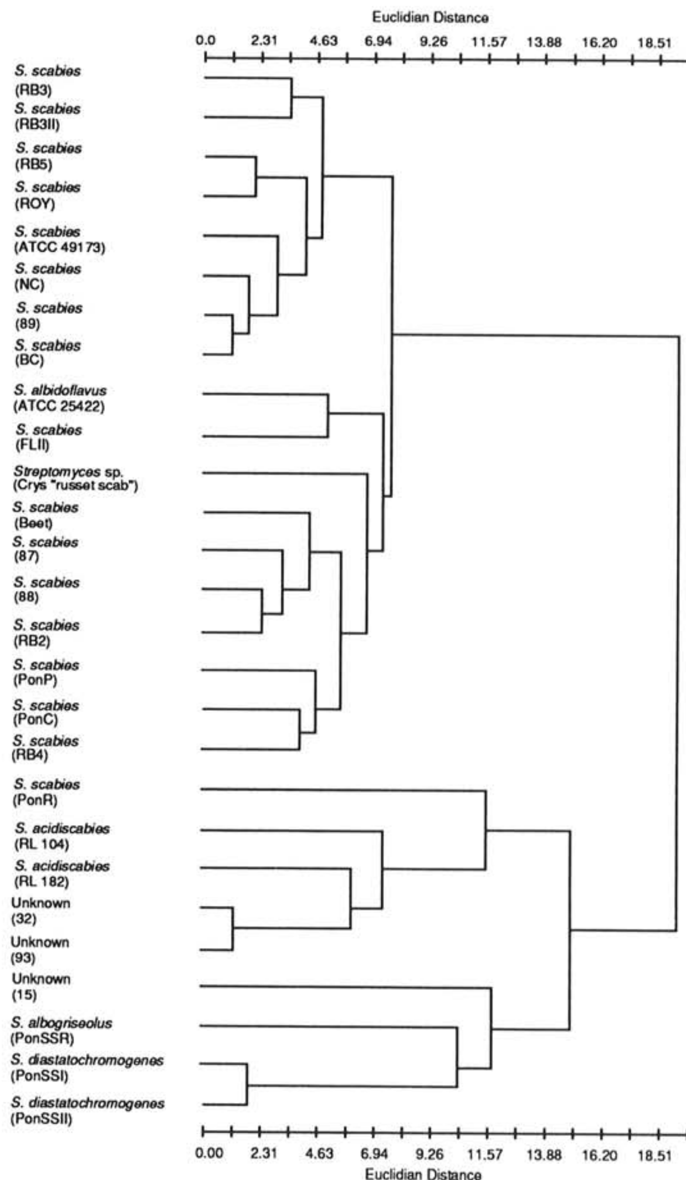

Fig. 2. Relationships among 20 pathogenic and 7 suppressive *Streptomyces* strains based on percent cellular fatty acid composition. Each entry is based on mean percent composition of fatty acids for 20 independent replicates.

example, strain PonR, identified as *S. scabies* based on taxonomic criteria (23), is only very weakly pathogenic in the greenhouse under conditions optimal for infection development. This strain is suppressive to pathogenic *S. scabies* and reduces disease levels after inoculation into field soil (26). In contrast with the taxonomic assignment of this strain to *S. scabies*, fatty acid analysis clusters PonR with the other pathogen-suppressive strains rather than with the pathogens. Results from fatty acid analysis also generally correspond with the groupings identified by Lorang et al. (28) who used a variety of biological criteria (inhibitory reactions including the lethal zygosis reaction, antibiotic production, and sensitivity to antibiotics) to develop groupings among 17 strains of *Streptomyces*. Groupings of these 17 *Streptomyces* strains based on fatty acid composition were identical to the biological groupings obtained using Lorang's criteria, with the minor exception that pathogenic strains RB3 and RB3II were placed in a different pathogen subgroup using Lorang's criteria than when using fatty acid analysis. The only difference between these two pathogen subgroups is the level of resistance of strains in the two groups to antibiotics produced by strains in other groups (28).

Production of the thaxtomin toxins by *Streptomyces* plays a key role in pathogenicity (17,18), and screening for thaxtomin production has been suggested as a simple strategy for distinguishing pathogenic and nonpathogenic strains in ecological studies. However, screening tests for thaxtomin production alone will provide only limited insight into the overall ecology and diversity of the *Streptomyces* community. In contrast, fatty acid analysis can be used to track the population dynamics of specific microbial strains in the environment and also can be used to quantify diversity within the *Streptomyces* community (5).

The pathogenic strains of *S. scabies* (not including the non-thaxtomin-producing suppressive strain PonR) formed one major group that was distinct from the suppressive and pathogenic strains of *S. acidiscabies*. Within the major pathogenic *S. scabies* cluster, there were two distinct subclusters related at a euclidean distance

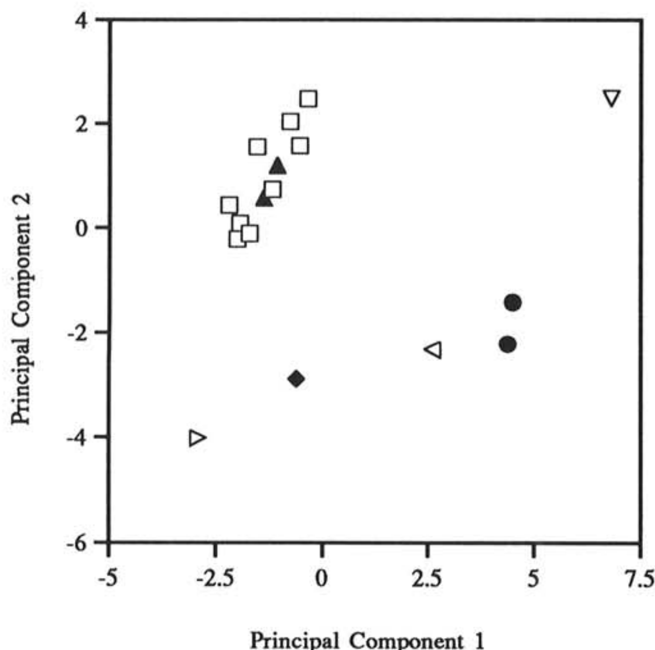

Fig. 3. Comparison of groups of 17 *Streptomyces* strains based on principal component analysis of fatty acids compared to groups based on inhibitory reactions, antibiotic production, and sensitivity to antibiotics. Groups based on inhibitory reactions, antibiotic production, and sensitivity to antibiotics are: group 1: Roy, NC, Beet, BC, RB2, RB4, RB5, PonC, PonP (*S. scabies* strains) (□); group 2: RB3 and RB3II (*S. scabies*) (▲); group 3: PonR (*S. scabies*) (◁); group 4: FLII (*S. scabies*, Florida strain) (♦); group 5: Crys "russet scab" (▷); group 6: PonSSI and PonSSII (*S. diastatochromogenes*) (●); and group 7: PonSSR (*S. albogriseolus*) (▽).

of 8.44. The presence here of two major clusters within the pathogen population is consistent with the findings of Paradis et al. (31), who found that among 31 strains of *Streptomyces* phenotypically similar to *S. scabies*, there were two main clusters. Based on the euclidean distance of 8.44 between the two major pathogen subclusters in this work, fatty acid analysis suggests that strains within these clusters are very closely related (33), despite the taxonomic assignment of strains within these clusters to distinct species. These results contrast with DNA-DNA hybridization studies that considered pathogenic *S. albidoflavus*, *S. scabies*, and *S. acidiscabies* strains. DNA-DNA hybridization studies showed a wide range of genetic variability among these groups and suggested a greater average relatedness between *S. scabies* and *S. acidiscabies* isolates than between *S. scabies* and *S. albidoflavus* (13). Further studies of the relationships between traditional taxonomic approaches, genetic analysis, fatty acid analysis, and ecological function of strains of *Streptomyces* in the soil environment are needed.

There is great potential for the use of suppressive *Streptomyces* spp. as biological control agents. Possible relationships among the collection of pathogen-antagonistic *Streptomyces* being investigated in different research groups are largely unknown. Intriguingly, the fatty acid profile of an *S. griseoviridis* strain isolated in Europe and currently sold as Mycostop (Agbio Development, Westminster, CO) for the biological control of a range of fungal plant pathogens is similar to that of the Minnesota suppressive strains investigated in this work. Specifically, Minnesota strain 15 and Mycostop were related at a euclidean distance of 8.6 (T. C. R. Ndowora, L. L. Kinkel, R. K. Jones, and N. A. Anderson, *unpublished data*). The extent to which potential suppressive strains from a wide geographic region represent a distinct subset of the total *Streptomyces* communities deserves further consideration.

A large number of *Streptomyces* spp. are pathogenic on potato (2,7,8,13) and other plant hosts. In our studies, we considered only *S. acidiscabies*, *S. scabies*, a russet scab pathogen of unknown taxonomic origin, and a representative of the *S. albidoflavus* group of pathogens (35). Future work should consider the differences in fatty acid composition among a greater range of pathogenic *Streptomyces* strains, including those from diverse plant hosts, to determine the utility of this technique for consistently distinguishing pathogenic from nonpathogenic strains and for discriminating among pathogen groups. Additionally, further work should expand the collection of both pathogenic and nonpathogenic strains of *Streptomyces* for which fatty acid data has been collected to further evaluate the relationships between fatty acid composition and traditional taxonomic criteria, geographic origin, genetic relatedness, and ecological traits of individual strains.

LITERATURE CITED

1. Adams, M. J., and Lapwood, D. H. 1978. Studies on the lenticel development, surface microflora and infection by common scab (*Streptomyces scabies*) of potato tubers growing in wet and dry soils. Ann. Appl. Biol. 90:335-343.
2. Archuleta, J. G., and Easton, G. D. 1981. The cause of deep-pitted scab of potatoes. Am. Potato J. 58:385-392.
3. Bonde, M. R., and McIntyre, G. A. 1968. Isolation and biology of *Streptomyces* sp. causing potato scab in soils below pH 5.0. Am. Potato J. 45: 273-278.
4. Bouzar, H., Jones, J. B., and Hodge, N. C. 1993. Differential characterization of *Agrobacterium* species using carbon-source utilization patterns and fatty acid profiles. Phytopathology 83:733-739.
5. Bowers, J. H., Kinkel, L. L., and Jones, R. K. Influence of disease-suppressive strains of *Streptomyces* on the native *Streptomyces* community in soil as determined by the analysis of cellular fatty acids. Can. J. Microbiol. In press
6. De Boer, S. H., and Sasser, M. 1986. Differentiation of *Erwinia carotovora* ssp. *carotovora* and *Erwinia carotovora* ssp. *atroseptica* on the basis of cellular fatty acid composition. Can. J. Microbiol. 32:796-800.
7. Doering-Saad, C., Kampfer, 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.

8. Faucher, E., Otrysko, B., Paradis, E., Hodge, N. C., Stall, R. E., and Beaulieu, C. 1993. Characterization of Streptomycetes causing russet scab in Quebec. Plant Dis. 77:1217-1220.
9. Faucher, E., Savard, T., and Beaulieu, C. 1992. Characterization of Actinomycetes isolated from common scab lesions on potato tubers. Can. J. Plant Pathol. 14:197-202.
10. Gitaitis, R. D., and Beaver, R. W. 1990. Characterization of fatty acid methyl ester content of *Clavibacter michiganensis* subsp. *michiganensis*. Phytopathology 80:318-321.
11. Graham, J. H., Hodge, N. C., and Morton, J. B. 1995. Fatty acid methyl ester profiles for characterization of glomalean fungi and their endomycorrhizae. Appl. Environ. Microbiol. 61:58-64.
12. Hayashida, S., Choi, M. Y., Nanri, N., Yokoyama, M., and Uematsu, T. 1989. Control of potato common scab with an antibiotic biofertilizer produced from swine feces containing *Streptomyces*. Agric. Biol. Chem. 53: 349-354.
13. Healy, F. G., and Lambert, D. H. 1991. Relationships among *Streptomyces* spp. causing potato scab. Int. J. Syst. Bacteriol. 41:479-482.
14. Hooker, W. J. 1981. Common scab. Pages 33-34 in: Compendium of Potato Diseases. W. J. Hooker, ed. American Phytopathological Society, St. Paul, MN.
15. Keinath, A. P., and Loria, R. 1989. Population dynamics of *Streptomyces scabies* and other Actinomycetes as related to common scab of potato. Phytopathology 79:681-687.
16. Keinath, A. P., and Loria, R. 1991. Effects of inoculum density and cultivar resistance on common scab of potato and population dynamics of *Streptomyces scabies*. Am. Potato J. 68:515-524.
17. King, R. R., Lawrence, C. H., and Calhoun, L. A. 1992. Chemistry of phytotoxins associated with *Streptomyces scabies* the causal organism of potato common scab. J. Agric. Food Chem. 40:834-837.
18. King, R. R., Lawrence, C. H., and Clark, M. C. 1991. Correlation of phytotoxin production with pathogenicity of *Streptomyces scabies* isolates from scab infected potato tubers. Am. Potato J. 68:675-680.
19. Kloepper, J. W., Schroth, M. N., and Miller, T. D. 1980. Effects of rhizosphere colonization by plant growth-promoting rhizobacteria on potato plant development and yield. Phytopathology 70:1078-1082.
20. Kuster, E. 1959. Outline of a comparative study of criteria used in characterization of the Actinomycetes. Int. Bull. Bacteriol. Nomencl. Taxon. 9:97-104.
21. Lambert, D. H., and Loria, R. 1989. *Streptomyces scabies* sp. nov. rev. Int. J. Syst. Bacteriol. 39:387-392.
22. Lambert, D. H., and Loria, R. 1989. *Streptomyces acidiscabies* sp. nov. Int. J. Syst. Bacteriol. 39:393-396.
23. Liu, D. 1992. Biological control of *Streptomyces scabies* and other plant pathogens. Ph.D. thesis. University of Minnesota, St. Paul.
24. Liu, D., and Anderson, N. A. 1992. Selecting isolates of *Streptomyces* suppressive to *Streptomyces scabies*. (Abstr.) Phytopathology 82:1108.
25. Liu, D., Anderson, N. A., and Kinkel L. L. 1994. Biological control of potato scab in field tests. (Abstr.) Phytopathology 84:1114.
26. Liu, D., Anderson, N. A., and Kinkel, L. L. 1995. Biological control of potato scab in the field with antagonistic *Streptomyces scabies*. Phytopathology 85:827-831.
27. Lorang, J. M., Anderson, N. A., Lauer, F. I., and Wildun, D. K. 1989. Disease decline in a Minnesota scab plot. Am. Potato J. 66:531.
28. Lorang, J. M., Liu, D., Anderson, N. A., and Schottel, J. A. 1995. Identification of potato scab inducing and suppressive species of *Streptomyces*. Phytopathology 85:261-268.
29. Manzer, F. E., McIntyre, G. A., and Merriam, D. C. 1977. A new potato scab problem in Maine. Life Sci. Agric. Exp. Stn. Tech. Bull. 85:3-17.
30. Menzies, J. D. 1959. Occurrence and transfer of a biological factor in soil that suppresses potato scab. Phytopathology 49:648-652.
31. Paradis, E., Goyer, C., Hodge, N. C., Hogue, R., Stall, R., and Beaulieu, C. 1994. Fatty acid and protein profiles of *Streptomyces scabies* strains isolated in Eastern Canada. Int. J. Syst. Bacteriol. 14:561-564.
32. Pridham, T. G., and Tresner, H. D. 1974. *Streptomycetaceae*. Pages 747-829 in: Bergey's Manual of Determinative Bacteriology. 8th ed. R. E. Buchanan and N. E. Gibbons, eds. The Williams and Wilkins Co., Baltimore.
33. Sasser, M. 1990. Identification of bacteria by gas chromatography of cellular fatty acids. Tech. Note 101. MIDI, Newark, DE.
34. Shirling, E. B., and Gottlieb, D. 1966. Methods for characterization of *Streptomyces* species. Int. J. Syst. Bacteriol. 16:313-340.
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.