Fatty Acid and Protein Profiles of *Streptomyces scabies* Strains Isolated in Eastern Canada

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Fatty acid composition was assessed for 31 pathogenic and nonpathogenic strains phenotypically related to *Streptomyces scabies* and isolated in eastern Canada. The profiles of these strains consisted of 12 to 17 fatty acids, most of which were saturated iso and anteiso acids. The 31 strains were clustered into two groups within a Euclidian distance of 25. Members in the first group were characterized by the predominance in their profile of the 16:0 acid palmitate. The second group was split into two subgroups at a Euclidian distance of 12. The 15:0 anteiso acid was the predominant fatty acid in strains of the first subgroup. Three acids (15:0 iso, 15:0 anteiso, and 16:0), each representing about 20% of the total fatty acid profile, characterized strains of the second subgroup. The protein profiles of some of those strains were also compared, and the genetic relatedness among the strains was estimated by DNA-DNA hybridization. Hybridization values suggested that two genetically diverse groups of strains are included in *S. scabies*. No correlation was established between fatty acid profiles and genetic clusters. No distinctive characteristics were associated with pathogenic strains.

Common scab of potato, found in all potato-growing areas of North America and Europe, is a disease which causes important economic losses in eastern Canada (12). The disease is characterized by superficial or deep corky lesions on tubers. Consequently, potato quality is severely affected, resulting in diminished marketable yields.

For many years, there was confusion as to the taxonomic identity of potato scab-inducing bacteria. Lambert and Loria (10, 11) helped to clarify the taxonomic position of these plant pathogens by demonstrating that *Streptomyces scabies*, a soil-borne actinomycete, was the predominant species associated with common scab. These investigators characterized *S. scabies* as having grey, smooth spores borne in spiral chains, producing melanin, and utilizing sucrose, L-arabinose, fructose, glucose, mannitol, raffinose, L-rhamnose, and D-xylose (11). However, numerous common scab-inducing organisms do not match the description of *S. scabies* (2, 3, 10).

High levels of genetic diversity have been observed for strains of *S. scabies*. DNA-DNA hybridization studies performed by Healy and Lambert (5) showed that the level of relatedness between two strains of this species could be as low as 20%. Their study also revealed that nonpathogenic streptomycetes, which share with *S. scabies* the same diagnostic properties, cannot be differentiated from pathogenic strains on the basis of DNA relatedness (5).

In a previous work, we characterized *Streptomyces* strains causing common scab of potato in Québec (3). Three pathogenic species were identified, but *S. scabies* was the predominant one. Several nonpathogenic strains, phenotypically similar to *S. scabies*, were also isolated. The purposes of this study were to (i) pursue the analysis of phylogenetic diversity among strains causing common scab of potato in eastern Canada and (ii) compare pathogenic and nonpathogenic strains phenotyp-

ically related to *S. scabies*. Three methods useful for determining the level of bacterial diversity among bacteria pathogenic for plants were used in this work: analysis of fatty acid profiles (1, 18), analysis of electrophoretic protein patterns (7, 22), and DNA-DNA hybridization studies (5).

MATERIALS AND METHODS

Bacterial strains and culture media. The bacterial strains used in this study are listed in Table 1. Bacteria were routinely propagated on yeast-malt extract (YME) medium (16). Streptomycetes were grown for 3 to 9 days in 125 ml of fatty acid-free Trypticase soy broth (BBL) prior to fatty acid analysis. For electrophoretic analysis of soluble proteins, YGM⁺ medium (8) was used.

Fatty acid analysis. Fatty acid analysis was performed on cultures in the mid-log phase of growth. Cultures were passed through cellulosic 0.45-µm-pore-size filters, and approximately 40 mg (wet weight) of cells was transferred to glass test tubes (13 by 100 mm) fitted with Teflon-lined caps for fatty acid extraction. Cellular fatty acids were extracted and derivatized to their methyl esters (FAMES) according to the protocol of Miller (15). For several strains, cellular fatty acids were extracted in duplicate to assess the reproducibility of the derivatization technique as well as the gas chromatographic system. Statistical analysis of FAME profiles was done with MIDI Library Generation System software (Microbial ID, Inc., Newark, Del.).

Extraction of soluble proteins. Soluble proteins were extracted from mycelia by the procedure of Guglielmi et al. (4), modified as follows. Bacterial spores were inoculated into 50 ml of liquid YGM⁺ medium and incubated for 24 to 48 h at 30°C on an orbital incubator (280 rpm). Cultures were centrifuged at 8,000 × g, and the pellets were washed once with 25 ml of buffer A (4); cells were resuspended in 7.5 ml of the same buffer. Bacterial suspensions were stored at -70°C for at least 24 h. Proteins were extracted from thawed cell suspensions by sonication for 1 min at an intensity of 35 to 40 on a Quigley-Rochester sonicator (model 2100), the addition of sodium

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TABLE 1. Bacterial strains used in this study

Bacterial strain(s)	Pathogenicity for potato tubers	Geographical origin	Reference
EF-5, EF-7, EF-11, EF-35, EF-46, EF-49, EF-50, EF-53, EF-54, EF-56, EF-57, EF-58, EF-59, EF-63, EF-64, EF-74, EF-80, EF-84, EF-90, EF-125, EF-127, EF-128, EF-132	+	Québec	3
OP-8, R90-8	+	Ontario	7
F89-19	+	New Brunswick	7
EF-2, EF-3, EF-15, EF-52, EF-73, EF-89	—	Québec	3

dodecyl sulfate (SDS) to a final concentration of 1%, and heating of the samples at 90°C for 3 min. Lysates were centrifuged in a microcentrifuge for 10 min; the supernatants were recovered and dialyzed overnight against distilled water. The protein concentrations in the dialyzed extracts were determined by the method of Rubin and Warren (17).

Protein electrophoresis. Samples containing 30 µg of extracted proteins and 12 µg of two reference proteins (phosphorylase *a* [molecular weight, 92,500] and soybean trypsin inhibitor [molecular weight, 21,500]) were loaded into the wells of an SDS-polyacrylamide gel. The stacking and resolving gels contained 4 and 14% polyacrylamide, respectively. Gel proteins migrated at 70 V until the bromophenol blue tracking dye reached the resolving gel; voltage was then increased to 140 V. Electrophoresis was conducted until the tracking dye reached the bottom of the gels. Gels were transferred for 2 h to a staining solution containing 0.25% Coomassie blue R-250 stain, 45% methanol, and 10% acetic acid. After destaining in an aqueous solution containing 45% methanol and 10% acetic acid, gels were dried between two sheets of Bio-Gel wrap (Biodesignt, Carmel, N.Y.).

Densitometric analysis of electrophoregrams. Dried, stained gels were scanned with a Bio-Rad video densitometer (model 620). Analysis of the protein electrophoregrams was performed with Bio-Rad 1-D analyst II software (version 3.10). After subtraction of the background and alignment of the reference proteins in different electrophoregrams, the position and the density of bands from different protein profiles were compared. Pearson's product-moment correlation coefficient (19) was applied to the data, and bacterial strains were clustered by the UPGMA method. The reproducibility of the method was evaluated by preparing protein extracts in duplicate and by calculating the correlation coefficient for replicated electrophoregrams of the same extracted protein.

DNA-DNA hybridization. Total DNA from *Streptomyces* strains was isolated by procedure 3 described by Hopwood et al. (6). The DNA reassociation experiments were performed as described by Stall et al. (20) but with the following modifications. The DNA was allowed to reassociate for 24 h at 64°C. The DNA of one strain of each of the protein groups and subgroups (EF-35, EF-54, and EF-64) was labeled by incorporating tritiated dCTP into sheared genomic DNA by the random primer method. Each labeled DNA was hybridized with sheared DNA of nine strains. Labeled double-stranded DNA was incorporated onto Whatman GF/C glass fiber filters, and radioactivity was quantified by liquid scintillation counting. The hybridization values for pairs of strains were means of the values from two experiments; for each experiment, two hybridization reactions were performed.

RESULTS

Fatty acid analysis. Bacterial FAME profiles can considerably change with culture age and culture conditions (18);

however, under the conditions used in this study, the reproducibility of the method used for fatty acid analysis was satisfactory. The FAME profiles of the streptomycetes consisted of 12 to 17 fatty acids, most of which were saturated iso or anteiso acids (Table 2). Quantitative differences were the basis for the designation of two groups of strains. The higher proportion (mean, 30.8%) of hexadecanoic acid, 16:0, synthesized by strains in group 1 was the primary factor for segregation of the population. Statistical differences in the relative ratios of the two structural isomers, 15:0 iso and 15:0 anteiso, facilitated the separation of the strains in group 2 into two subgroups, A and B.

On the basis of the MIDI dendrogram software program, the population was linked within a Euclidian distance of 25, indicating that all the strains were likely to be members of the same genus (Fig. 1) (14). Members of group 1, EF-2, EF-53, R90-8, F89-19, and OP-8, clustered at a Euclidian distance of approximately 10. The second group was split into two subgroups, A and B, at a Euclidian distance of 12. The ratios of two isomers, 15:0 iso and 15:0 anteiso, varied inversely, 0.73 and 1.09 for subgroups A and B, respectively. The mean percentages of two dissimilar acids, 16:0 and 17:0 anteiso, further distinguished the subgroups. Pathogenic and non-pathogenic strains were present in all groups and subgroups (Fig. 1 and Table 2).

Whole-cell protein analysis. On the basis of the analysis of whole-cell soluble protein electrophoregrams, UPGMA divided the *Streptomyces* strains into two distinct groups, with a correlation coefficient of 0.73 (Fig. 2). Strains of the first group (EF-2, EF-35, and R90-8) were closely related, since they

TABLE 2. Fatty acid compositions of pathogenic and nonpathogenic strains phenotypically related to *S. scabies*

Fatty acid ^a	% (SD) of fatty acid in:		
	Group 1 (n = 8)	Group 2	
		Subgroup A (n = 23)	Subgroup B (n = 9)
13:0 iso	1.33 (0.16)	<1%	1.06 (0.18)
14:0 iso	3.13 (1.0)	2.17 (0.37)	2.67 (0.33)
14:0	4.59 (1.0)	1.03 (0.10)	1.67 (0.34)
15:0 iso	11.20 (1.94)	17.46 (3.14)	20.89 (2.03)
15:0 anteiso	15.30 (0.97)	23.77 (2.21)	19.21 (1.65)
15:0	2.55 (0.53)	1.31 (0.20)	1.35 (0.30)
16:0 iso	7.33 (1.71)	10.79 (1.68)	9.34 (0.53)
16:1 cis 9	12.28 (2.55)	3.64 (0.64)	4.45 (1.42)
16:0	30.78 (2.81)	15.01 (0.64)	19.74 (2.18)
17:1 iso	1.63 (0.71)	2.66 (0.65)	2.62 (0.57)
17:1 anteiso	<1%	1.57 (0.46)	<1%
17:0 iso	2.68 (0.84)	8.18 (1.27)	8.35 (1.05)
17:0 anteiso	4.58 (0.94)	10.80 (1.43)	6.66 (1.22)

^a Fatty acids accounting for <1% have been omitted.

According to the DNA-DNA hybridization data, *S. scabies* strains could thus be divided into two genetic clusters. Genetic cluster 1 included strains EF-52, EF-54, and EF-56, whereas genetic cluster 2 included strains EF-46, EF-64, EF-73, EF-2, EF-35, and R90-8.

In this study, the fatty acid and protein compositions of pathogenic and nonpathogenic strains that were phenotypically

Strain	% Hybridization with:		
	EF-35	EF-54	EF-64
Protein group 1			
EF-2	115	59	111
EF-35	100	61	87
R90-8	100	67	84
Protein group 2 subgroup A			
EF-52	60	92	55
EF-54	50	100	67
EF-56	21	99	37
Protein group 2 subgroup B			
EF-46	87	58	77
EF-64	92	61	100
EF-73	93	60	111

DNA-DNA hybridization. DNA from one strain each of protein group 1 (EF-35), protein group 2 subgroup A (EF-54), and protein group 2 subgroup B (EF-64) was hybridized to DNAs from nine *S. scabiei* strains (Table 3). The levels of relatedness between DNA from EF-35 and DNAs from protein group 1 strains and from protein group 2 subgroup B strains ranged from 100 to 115% and from 87 to 93%, respectively. A lower level of relatedness was found between DNA from EF-35 and DNAs from strains of protein group 2 subgroup A (average, 44%). Like strain EF-35, strain EF-64 shared a high level of DNA relatedness with strains of protein group 1 and of protein group 2 subgroup B (average, 94%) and a low level of DNA relatedness with strains of protein group 2

related to *S. scabies* were compared, and the genetic relatedness among these strains was evaluated by DNA-DNA hybridization. Atypical potato scab-inducing strains and *S. acidiscabies* strains were not included in this study. Although all strains used in this study shared phenotypic properties and were all isolated in eastern Canada, DNA-DNA hybridization studies showed that the population of *S. scabies* is composed of two distinct groups of microorganisms, which may represent two *Streptomyces* species. Healy and Lambert (5), who analyzed the levels of DNA relatedness among other *S. scabies* strains, also concluded that *S. scabies* strains show more diversity than a conventional species. This conclusion suggests that potato scab can be induced by several species, some of which exhibit phenotypic characteristics associated with *S. scabies* and some, such as *S. acidiscabies*, that can be phenotypically differentiated from *S. scabies* (3, 9). Healy and Lambert (5) also showed that pathogenic strains of *S. scabies* cannot be differentiated from nonpathogenic strains on the basis of DNA relatedness (5). This study supports their conclusion.

DNA-DNA hybridization studies allowed the division of *S. scabies* strains into genetic clusters 1 and 2. No correlation was found between fatty acid profiles and genetic clusters. However, there was a positive correlation between genetic cluster 1 and protein group 2 subgroup A. Genetic cluster 2 comprised members of protein group 1 and members of protein group 2 subgroup B.

A group of strains which was included in genetic cluster 1 had protein and fatty acid profiles (fatty acid group 1 and protein group 1) clearly different from those of the other *S. scabies* strains. Even though this group of strains shared a high level of DNA relatedness with other members of genetic cluster 1, they synthesized different proteins and FAMES under the growth conditions used in this work.

The 31 strains tested had FAME profiles similar to those previously reported for streptomycetes (21). No distinctive patterns were associated with the protein and fatty acid profiles of pathogenic strains. With fatty acid analysis as the sole criterion, some pathovars of *Xanthomonas campestris* cannot be differentiated (1). Similarly, we conclude that fatty acid and whole-cell protein analyses are unable, independently or in concert, to differentiate pathogenic and nonpathogenic strains phenotypically related to *S. scabies*.

Pathogenicity mechanisms of organisms causing common scab are poorly understood. Whole-cell protein data suggested that few proteins are essential to pathogenicity or that the proteins involved in pathogenesis were not produced under the culture conditions used in this study. Toxins termed thaxtomins, however, have been found associated with infected potato tubers (13). These toxins are produced by pathogenic strains in plants but are not produced in YME⁺ medium (8). It is possible that at least some of the enzymes involved in thaxtomin synthesis have not been detected by protein electrophoresis.

There is no rapid test to ascertain the pathogenicity of a *Streptomyces* strain. Pathogenic strains cannot be recognized by physiological or morphological traits, protein electrophoresis, or fatty acid analyses. The production of thaxtomins, however, could be useful as a diagnostic assay but not as a taxonomic parameter, since these phytotoxins are not produced solely by strains of *S. scabies* (8, 9).

ACKNOWLEDGMENTS

We thank P. Béchar and L. Gaudreau for critical reading of the manuscript and C. H. Lawrence for the gift of bacterial strains.

This work was supported by a grant from the Natural Sciences and Engineering Research Council of Canada.

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