Comparison of Whole-Cell Fatty Acid Compositions in Intraspecific Groups of *Rhizoctonia solani* AG-1

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ABSTRACT

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Gas chromatography and integrated computer software (Microbial Identification System, Microbial ID, Inc., Newark, DE) were used to identify and compare fatty acid compositions among isolates representing three intraspecific groups of *Rhizoctonia solani* anastomosis group 1 (AG-1). The major fatty acids found, constituting 94–96% of the C9-C20 fatty acids present, were linoleic (18:2 cis 9, 12), oleic (18:1 cis 9), and palmitic (16:0). Smaller amounts of nine other fatty acids were consistently identified in preparations from 2- and 4-day-old cultures. Library entries were generated for the intraspecific groups AG-1 IA, AG-1 IB, and AG-1 IC. A dendrogram of these library entries showed that AG-1 IA and AG-1 IB are very closely related in fatty acid composition, whereas AG-1 IC is different. Intraspecific group libraries challenged with independent isolates could be used to identify most isolates of AG-1 IB and AG-1 IC but not AG-1 IA. Most of the isolates of AG-1 IA were identified as AG-1 IB.

Additional keywords: sheath blight, web blight.

Rhizoctonia solani Kühn (teleomorph = *Thanatephorus cucumeris* (A.B. Frank) Donk) contains a diverse grouping of fungi that exhibits a wide and sometimes overlapping range of pathogenicity (1). Plant pathologists and diagnosticians continue to be interested in rapid and accurate identification of isolates of *Rhizoctonia* spp. because they represent an important group of fungi frequently associated with diseased plants.

Differentiation of isolates into meaningful groupings has evolved from the observation that similar isolates exhibit a vegetative compatibility mechanism called anastomosis (30,31). Anastomosis groups (AGs) of *R. solani* represent the primary means used to differentiate isolates below the species level. Currently, 10 AGs of *R. solani* are recognized (2,4,14–16).

Ogoshi (14,15) proposed further subspecific division of AG-1 into intraspecific groups (ISGs) based on pathogenicity: sasakii type (IA), web blight type (IB), and a microsclerotial type (IC) recovered from buckwheat and tare soil from sugar beet fields (8). These ISGs also differ in their cultural appearance (9,15). Ogoshi's IA, IB, and IC from Japan correspond to Sherwood's (19) types 2, 1, and 3, respectively, from the United States (14).

Physiological and genetic characteristics also have been used to differentiate the ISGs of AG-1. These include isozyme patterns (12,13), DNA base composition (10), DNA base sequence homology (11,25), and ribosomal DNA restriction fragment length polymorphisms (RFLP) (26). Host-range studies have been less successful in differentiating isolates of AG-1 into ISGs because members of this AG often attack a broad and overlapping range of host plants, especially under inoculated conditions (5–9,15, 24,28,29).

Unique whole-cell fatty acid profiles have been used successfully to differentiate the ISGs of *R. solani* AG-2-2 and pathogenically distinct populations in AG-3 affecting potato and tobacco (22,23). The purpose of our research was to use the Microbial Identification System (MIS; Microbial ID, Inc., Newark, DE) to examine fatty acid composition in isolates of *R. solani* AG-1 with respect to its capacity to differentiate ISGs of AG-1. Preliminary results have been published (20,21).

MATERIALS AND METHODS

Source of isolates. We compared fatty acid compositions of 20 isolates each from *R. solani* ISGs AG-1 IA, AG-1 IB, and AG-1 IC (Table 1). Isolates of *R. solani* AG-1 IA were collected as sasakii-type sclerotia from soils in which crops of rice (*Oryza sativa* L.) or soybean (*Glycine max* (L.) Merr.) had been grown (3). Most of the AG-1 IB isolates were collected from soybean leaves with web blight symptoms (9,29). Cultures were derived from microsclerotia, hymenia, or leaf lesions. Isolates of *R. solani* AG-1 IC were collected from diverse hosts, including eight from sugar beet or sugar beet tare soil (28) (Table 1). All isolates were maintained on potato-dextrose agar (Difco Laboratories, Detroit, MI) amended with 50 mg/L each of streptomycin sulfate and penicillin G (PDA+).

Cellular fatty acid analyses. Cultures were grown on PDA+ at 28 C in the dark for 2 and 4 days. For 2-day-old cultures, a 32-cm^2 mycelial mat was removed, weighed, and analyzed as previously described (21). Preliminary data from 32-cm^2 mycelial mats of 4-day-old cultures revealed quantities of 18:2 cis 9, 12 that exceeded manufacturers specifications for the gas chromatography column. Therefore, the mycelial fresh weight was standardized to approximately 0.5 g for 4-day-old cultures. We were able to use entire 32-cm^2 mycelial mats from 2-day-old cultures. The area of mycelial mats from 4-day-old cultures was approximately one-half the area of those from 2-day-old cultures.

Fatty acid methyl ester (FAME) compositions were determined for each isolate. Each extraction and analysis was conducted with three replicate plates per run. Analysis of variance (ANOVA; SAS Institute, Cary, NC) was performed on percent FAME compositions. Mean separation was accomplished by the Waller-Duncan

K-ratio t test ($K = 100$, $P = 0.05$). Principal component analysis (SAS Institute) was performed on mean percent FAME compositions (six samples per isolate). Only the first three principal components were used.

Library generation. Library Generation Software (Microbial ID, Inc.) was used to develop two types of fatty acid libraries: an isolate library and a population (ISG) library. Each library entry was a set of data points representing the mean percent composition of each fatty acid found in the samples used to create that entry.

Isolate library entries, used to compare isolates within the ISGs of *R. solani* AG-1, were based on the mean percent composition of each fatty acid from the six replicate samples of that isolate. Fatty acids used to create an isolate library entry were required to be reproducibly detectable in the isolate. A fatty acid was included in a library entry if the product of its mean percent composition multiplied by the percentage of total samples that contained the fatty acid exceeded 0.25 (22). This prevented fatty acids represented in very low concentrations or in peaks detected only once or twice from being included in an isolate library entry.

Relative similarities among isolate library entries were determined by cluster analysis. A resemblance matrix based on Euclidean-distance coefficients was computed from the pairwise comparisons of each isolate entry with every other isolate entry based on qualitative and quantitative differences in fatty acid composition. The Euclidean distance was the distance in two-dimensional space between isolate entries when their fatty acid compositions were compared. A dendrogram was constructed by the unweighted pair-group method with arithmetic averages and was used to depict relatedness of pairs of entries.

Two sets of population (ISG) library entries were developed for each of AG-1 IA, AG-1 IB, and AG-1 IC for comparison among the ISGs. In the first set, each entry in the population library represented the mean of fatty acid compositions from 60 samples (10 isolates with six replicate samples per isolate). For the second set of library entries, 10 additional isolates per ISG were included, yielding 120 samples (20 isolates with six replicate samples per isolate). A similarity index (value between 0.0 and 1.0) was calculated to express how nearly the profile of a given sample matched that of the population (ISG) library entry (21). This index reflects the sample's distance in n-dimensional space (Gaussian distance) from the mean profile of the closest library entry. Dendrograms were constructed to compare population library entries of AG-1 IA, AG-1 IB, and AG-1 IC.

TABLE 1. Source of isolates

Isolate	AG/ISG[x]	Host/origin	Reference ID	Source[y]
1-1-001[z]	AG-1 IA	Rice, TX	Belmar F-3(2)	3
1-1-002[z]	AG-1 IA	Rice, TX	Belmar F-4(1)	3
1-1-003[z]	AG-1 IA	Rice, TX	Belmar F-7A(2)	3
1-1-004[z]	AG-1 IA	Rice, TX	Belmar F-7B(2)	3
1-1-005[z]	AG-1 IA	Rice, TX	Belmar F-7B(3)	3
1-1-006[z]	AG-1 IA	Rice, TX	Belmar F-7C(1)	3
1-1-007[z]	AG-1 IA	Rice, TX	Belmar F-17(3)	3
1-1-008[z]	AG-1 IA	Rice, TX	Belmar F-22-1B	3
1-1-009[z]	AG-1 IA	Rice, TX	Belmar F-22-2B	3
1-1-010[z]	AG-1 IA	Rice, TX	Belmar F-22-4A	3
1-1-020	AG-1 IA	Rice, TX	Belmar F-32-3A	3
1-1-021	AG-1 IA	Rice, TX	Belmar F-32-T7	3
1-1-022	AG-1 IA	Rice, TX	Belmar F-33-4A	3
1-1-023	AG-1 IA	Rice, TX	Belmar F-33-6A	3
1-1-025	AG-1 IA	Rice, TX	Belmar F-38-5A	3
1-1-026	AG-1 IA	Rice, TX	Belmar F-38-6A	3
1-1-027	AG-1 IA	Rice, TX	Belmar F-39-11B	3
1-1-028	AG-1 IA	Rice, TX	Belmar F-39-3B	3
1-1-029	AG-1 IA	Rice, TX	Jones NGW	3
1-1-031	AG-1 IA	Rice, TX	Jones HT-1A	3
1-2-001[z]	AG-1 IB	Soybean, TX	Jones SLL-1A	3
1-2-002	AG-1 IB	Soybean, TX	Jones SLL-1B	3
1-2-003[z]	AG-1 IB	Soybean, TX	Jones SLL-1C	3
1-2-004[z]	AG-1 IB	Soybean, TX	Jones SMS-2	3
1-2-005	AG-1 IB	Soybean, TX	Jones SMS-3	3
1-2-006	AG-1 IB	Soybean, TX	Jones SMS-4	3
1-2-007[z]	AG-1 IB	Soybean, TX	Jones SMS-5	3
1-2-008	AG-1 IB	Soybean, TX	Jones SMS-6	3
1-2-009[z]	AG-1 IB	Soybean, TX	Jones SMS-8	3
1-2-010[z]	AG-1 IB	Soybean, TX	Jones SMS-9	3
1-2-011[z]	AG-1 IB	Soybean, TX	Jones SMS-10	3
1-2-012[z]	AG-1 IB	Soybean, TX	Jones SHT-1	3
1-2-013[z]	AG-1 IB	Soybean, NC	Echandi-NC	3
1-2-014[z]	AG-1 IB	Poa, PA	ATCC 66150	2
1-2-016	AG-1 IB	Soybean, LA	Crowley-2	2
1-2-017	AG-1 IB	Soybean, LA	Crowley-3	2
1-2-018	AG-1 IB	Soybean, LA	Waterway-1	2
1-2-019	AG-1 IB	Soybean, LA	Cameroon-1	2
1-2-020	AG-1 IB	Soybean, LA	Lake Arthur-1	2
1-2-021	AG-1 IB	Soybean, LA	Atchafalaya-1	2
1-3-001[z]	AG-1 IC	Pine, Canada	ATCC 42128	3
1-3-002[z]	AG-1 IC	Sugar beet, MN	85-11-3R	5
1-3-003[z]	AG-1 IC	Sugar beet, MN	87-16-B	5
1-3-004	AG-1 IC	Pine, Canada	ATCC 42128	4
1-3-005[z]	AG-1 IC	Soil, Japan	ATCC 66155	2
1-3-006	AG-1 IC	Pine, Canada	ATCC 42128	2
1-3-007[z]	AG-1 IC	Lima bean, NY	Dillard 322	2
1-3-008[z]	AG-1 IC	Lima bean, NY	Dillard 332	2
1-3-009[z]	AG-1 IC	Lima bean, NY	Dillard 327	2
1-3-011[z]	AG-1 IC	White spruce	Parmeter C65	1
1-3-012[z]	AG-1 IC	Cauliflower	ATCC 13248	1
1-3-014[z]	AG-1 IC	Rice	Parmeter S257	1
1-3-015	AG-1 IC	Pine, Canada	ATCC 42128	5
1-3-016	AG-1 IC	Sugar beet, MN	103-4 Jac 638	5
1-3-017	AG-1 IC	Sugar beet, MN	104-4 Jac 69	5
1-3-018	AG-1 IC	Sugar beet, MN	104-4 Jac 2116	5
1-3-019	AG-1 IC	Sugar beet, MN	302-2 Jac 828	5
1-3-020	AG-1 IC	Sugar beet, MN	4-3-1 402	5
1-3-021	AG-1 IC	Sugar beet, MN	104-4 Jac 2117	5
1-3-025	AG-1 IC	Fig, LA	CMI 61796	1

[x] Anastomosis group/intraspecific group.

[y] 1 = G. C. Adams, Mich. St. Univ., East Lansing; 2 = G. T. Berggren, La. St. Univ., Baton Rouge; 3 = R. K. Jones, Univ. Minn., St. Paul; 4 = R. Vilgalys, Duke Univ., Durham, NC; 5 = C. E. Windels, Univ. Minn., Crookston.

[z] Denotes original 10 isolates from each ISG of AG-1 analyzed for fatty acid composition, at both 2 and 4 days of growth.

RESULTS

Cellular fatty acid analysis. Twelve fatty acids were present in 2- and 4-day-old cultures, grown on PDA+, of isolates representing all three ISGs of *R. solani* AG-1 (Tables 2 and 3). Three of these fatty acids (linoleic-18:2 cis 9, 12; oleic-18:1 cis 9; and palmitic-16:0) comprised 94.1–95.5% of the cellular fatty acids identified. Other fatty acids present in these isolates included: myristic (14:0), pentadecanoic (15:0), 2-hydroxypalmitic (16:0 2OH), palmitoleic (16:1 cis 9), heptadecanoic (17:0), 9-heptadecenoic (17:1 cis 9), stearic (18:0), the dimethylacetyl derivative of 11-octadecenoic acid (18:1 cis 11 dma), and an unknown fatty acid with an equivalent chain length (ECL) of 18.201 (21). The ECL was derived from its relative retention time compared to that of straight chain saturated fatty acids contained in the calibration standard (21).

Although the fatty acid compositions of the isolates were qualitatively similar, quantitative differences were observed. Tests for additivity showed independence in all fatty acids except 18:2 cis 9, 12. ANOVA for 18:2 cis 9, 12 with mean separations by Waller-Duncan's method ($K = 100$, $P = 0.05$) showed no difference in mean separation of populations between transformed [square root$(100 - X)$] and untransformed data (*data not shown*). Therefore, untransformed data were used in statistical analyses. ANOVA showed no effect of experimental runs (run 1 versus run 2) except in fatty acids 15:0 and 17:1 cis 9. Residual plots for each of these fatty acids indicated random variability. Therefore, data were combined for further statistical analyses.

Variation between and within ISGs. ANOVA indicated there were significant differences in fatty acid composition ($P = 0.001$) among isolates within each ISG at 2 or 4 days. In addition, significant differences ($P = 0.05$) were observed among ISGs for each of the 12 fatty acids detected at 2 days (Table 2) and for 10 of the 12 fatty acids detected at 4 days (Table 3).

TABLE 2. Percent composition of cellular fatty acids identified in 2-day-old cultures of intraspecific groups (ISGs) of *Rhizoctonia solani* anastomosis group 1 (AG-1)

	Fatty acids (%)											
ISG	14:0	15:0	16:1 cis 9	16:0	17:1 cis 9	17:0	16:0 2OH	18:2 cis 9, 12	18:1 cis 9	18:0	ECL 18.201	18:1 cis 11 dma
AG-1 IA	0.08 c[z]	0.35 c	0.91 a	8.52 b	0.96 b	0.19 c	0.05 b	76.78 b	10.15 b	0.83 b	0.61 b	0.54 a
AG-1 IB	0.28 b	0.42 b	0.88 b	8.69 a	0.78 c	0.27 b	0.07 b	77.77 a	9.04 c	0.93 a	0.46 c	0.37 b
AG-1 IC	0.31 a	0.97 a	0.57 c	8.73 a	1.16 a	0.56 a	0.27 a	73.45 c	11.88 a	0.91 a	0.70 a	0.51 a

[z]Mean of 10 isolates with six replicate extractions per isolate, grown on potato-dextrose agar for 2 days. Values in each column followed by different letters are significantly different, Waller-Duncan *K*-ratio *t* test, $P = 0.05$.

TABLE 3. Percent composition of cellular fatty acids identified in 4-day-old cultures of intraspecific groups (ISGs) of *Rhizoctonia solani* anastomosis group 1 (AG-1)

	Fatty acids (%)											
ISG	14:0	15:0	16:1 cis 9	16:0	17:1 cis 9	17:0	16:0 2OH	18:2 cis 9, 12	18:1 cis 9	18:0	ECL 18.201	18:1 cis 11 dma
AG-1 IA	0.10 b[z]	0.88 b	0.72 a	8.37 b	0.95 b	0.48 a	0.04 c	77.10 b	9.52 b	1.13 b	0.29 a	0.42 a
AG-1 IB	0.27 a	0.89 b	0.69 a	8.09 c	0.88 c	0.48 a	0.09 b	77.87 a	8.75 c	1.29 a	0.29 a	0.28 b
AG-1 IC	0.29 a	1.19 a	0.62 b	8.52 a	1.09 a	0.48 a	0.22 a	72.99 c	12.94 a	1.09 c	0.29 a	0.28 b

[z]Mean of 10 isolates with six replicate extractions per isolate, grown on potato-dextrose agar for 4 days. Values in each column followed by different letters are significantly different, Waller-Duncan *K*-ratio *t* test, $P = 0.05$.

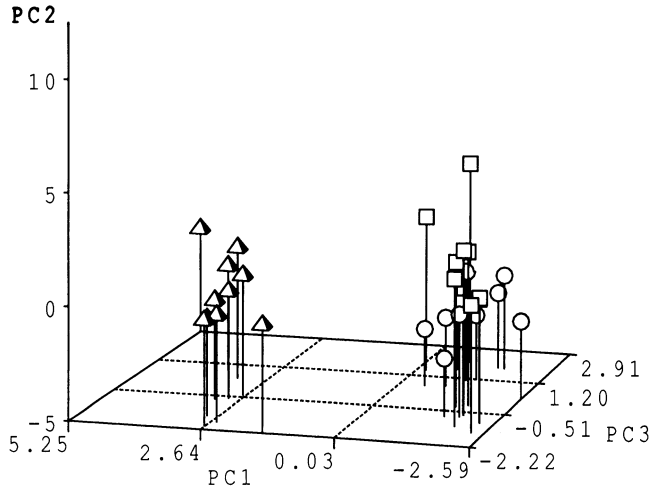

Fig. 1. Plot of the first three principal components (PC1, PC2, PC3) derived from the fatty acid percent composition (of all detected fatty acids) of *Rhizoctonia solani* anastomosis group 1 (AG-1) isolates, representing AG-1 IA (○), AG-1 IB (□), and AG-1 IC (△). The original 10 isolates of each intraspecific group grown on potato-dextrose agar for 2 days were used.

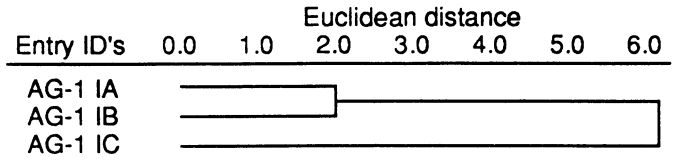

Fig. 2. Dendrogram of library entries of *Rhizoctonia solani* anastomosis group 1 (AG-1) populations, representing AG-1 IA, AG-1 IB, and AG-1 IC, based on the analysis of percent fatty acid composition of the original 10 isolates for each intraspecific group grown on potato-dextrose agar for 2 days.

Principal component analysis, based on all 12 fatty acids detected in 2-day-old cultures, confirmed the distinct separation of isolates representing AG-1 IC from isolates representing AG-1 IA and AG-1 IB (Fig. 1). The first three of 12 principal components (PC1, PC2, and PC3) of this analysis accounted for 78.0% of the variation in the data. PC1 accounted for 44.3% of the variability and PC2 accounted for 18.1% of the variability. Similar results were observed with the 4-day data.

Library generation. A fatty acid library for each ISG was created from 60 samples (10 isolates, six samples each). Nine of 12 fatty acids were reproducibly detected and met the quality threshold criteria (0.25). These nine fatty acids were used in the creation of all three ISG libraries. They included 15:0, 16:1 cis 9, 16:0, 17:1 cis 9, 18:2 cis 9, 12, 18:1 cis 9, 18:0, ECL 18.201, and 18:1 cis 11 dma. Qualitative differences in the presence of specific fatty acids also were used in generating libraries. These included 14:0 (used in AG-1 IB and AG-1 IC) and 17:0 (used in AG-1 IC). Fatty acid 16:0 2OH was present in all 10 isolates of AG-1 IC but at a percent below the quality threshold.

A comparison of the fatty acid profile of each sample with a library made exclusively from samples of that particular ISG produced similarity indices ranging from 0.821 to 0.997 for AG-1 IA, 0.852 to 0.993 for AG-1 IB, and 0.815 to 0.993 for AG-1 IC. The similarity index indicated how closely the fatty acid profile of a given sample matched the fatty acid composite of its library.

An AG-1 library was generated that contained three ISGs (IA, IB, and IC) as entries. Each entry was a fatty acid composite of 60 2-day-old samples from the respective populations. Fifty-nine of the AG-1 IB samples and 54 of the AG-1 IC samples had higher similarity indices to their own library entry than to any other library entry (i.e., were correctly identified). Only 25 of 60 AG-1 IA samples were correctly identified as AG-1 IA. The remaining samples were identified as AG-1 IB.

A dendrogram of the AG-1 ISG library entries (2-days growth) of AG-1 IA, AG-1 IB, and AG-1 IC showed that AG-1 IA and AG-1 IB were very closely related to each other with a Euclidean distance of 1.95 (Fig. 2). The AG-1 IC entry was less related to the AG-1 IA and AG-1 IB library entries with a Euclidean distance of 6.12. A dendrogram of the AG-1 ISG library entries (4-days growth) showed similar results; AG-1 IA and AG-1 IB clustered with a Euclidean distance of 1.50, and AG-1 IC was more distinct with a Euclidean distance of 6.20 (*data not shown*).

Additional isolates analyzed. Because of the difficulty in correctly identifying isolates of AG-1 IA, an attempt was made to improve the AG-1 library by adding samples. Ten additional isolates (Table 1) of AG-1 IA, AG-1 IB, and AG-1 IC were analyzed, compared with, and then added to the original library. As before, each isolate entry was represented by six replicate samples.

Cluster analysis of the library isolates and the additional isolates indicated that the level of variation among isolates was similar to that among the initial 10 isolates. The initial 10 isolate entries of ISGs IA, IB, and IC clustered at Euclidean distances of 3.60, 7.12, and 6.33, respectively. When 10 additional isolate entries

were included (total of 20 isolates), they clustered with Euclidean distances of 3.92, 6.33, and 6.87, respectively.

An AG-1 ISG library was generated that contained three ISGs (IA, IB, and IC) as entries with all 20 isolates of each ISG. Each entry was a fatty acid composite of 120 2-day-old samples from the respective populations. Ninety-five of the AG-1 IB samples and 100 of the AG-1 IC samples had higher similarity indices to their own library entry than to any other library entry (i.e., were correctly identified). Only 56 of 120 AG-1 IA samples were correctly identified as AG-1 IA. A dendrogram of the AG-1 ISG library, constructed with all 60 isolates (320 samples), revealed a very close relationship between the AG-1 IA and AG-1 IB ISG entries, which clustered with a Euclidean distance of 0.90 (*data not shown*). The AG-1 IC entry was more distinct from these ISGs and clustered with a Euclidean distance of 4.16.

Isolate identification. A collection of isolates of ATCC 42128 (AG-1 IC), obtained from multiple sources, was analyzed in an attempt to determine the ability of the AG-1 IC library to confirm isolate identity. Twenty-four of 24 samples of ATCC 42128 from isolates 1-3-001, 1-3-004, 1-3-006, and 1-3-015 (Table 1) were identified as isolate 1-3-001 (ATCC 42128) by the 2-day/10-isolate library (which contained only 1-3-001), with similarity indices of 0.778–0.996.

DISCUSSION

The fatty acid compositions of isolates of AG-1 IA and AG-1 IB were very similar, as indicated by principal component and cluster analyses. Additional isolates did not improve the ability to differentiate these two ISGs. The 10-isolate library identified 76% of the samples to the correct ISG, whereas the 20-isolate library only identified 70% of the samples correctly. The majority of the misidentifications involved AG-1 IA misidentified as AG-1 IB.

Although fatty acid analysis was not useful in differentiating isolates of AG-1 IA and AG-1 IB, morphological and pathological differences do provide a means of distinguishing these ISGs (9,15, 19). This distinct separation of AG-1 IA (sasakii type) from AG-1 IB (web blight type) has been recognized since 1953 (6). Differentiation includes recognition of disease signs, i.e., sasakii-type sclerotia versus microsclerotia produced on hosts, and appearance of the isolates in culture. The guanine-plus-cytosine content of isolates of AG-1 IA and AG-1 IB is quite similar (10). Isolates of AG-1 IA and AG-1 IB, including many of those examined in this study, could not be differentiated by isozyme analysis nor by RFLP analysis, but isolates of AG-1 IC were distinct according to these methods (12). These results suggest a closer affinity between the sheath blight pathogen and the web blight pathogen than has been recognized previously.

Isolates from different hosts, classified as AG-1 IC, had similar fatty acid profiles in a 10-isolate library (similarity indices of 0.815–0.993) but were distinct from isolates of AG-1 IA and AG-1 IB. Principal component and cluster analyses at both 2- and 4-days growth confirmed the ability to distinguish isolates of AG-1 IC from isolates of AG-1 IA and AG-1 IB.

A collection of cultures, from multiple sources, of ATCC 42128 (AG-1 IC) were correctly identified when compared with the 2-day/10-isolate library. The ability of fatty acid libraries to confirm isolate identity previously was demonstrated for isolates of AG-1 IA (21) and isolates within AG-2-2 (22). Isolate ATCC 42128 was obtained originally from a forest-tree nursery in Canada. Isolates belonging to AG-1 IC are pathogens in forest-tree nurseries, where they cause disease on young seedlings (17,18,27).

Isolates of *R. solani* AG-1 from sugar beet and sugar beet tare soil in Minnesota (1-3-002, 1-3-003, 1-3-016 through -021) clustered with isolate 1-3-005 of AG-1 IC (ATCC 66155) recovered from sugar beet tare soil in Japan. These isolates produced typical salt-and-pepper sclerotia (19) on media and were characterized as AG-1 IC by fatty acid analysis. Isolates of AG-1 (microsclerotial type) affecting lima beans in New York (5) also were examined. These isolates produced characteristic salt-and-pepper sclerotia on media and were identified as AG-1 IC.

Exner (6) characterized isolates of *R. solani* causing a disease of fig (*Ficus carica* L.) as *Pellicularia filamentosa* f. sp. *microsclerotia* (web blight type) and *P. f.* f. sp. *timsii* (new description). Both forma speciales have subsequently been reduced to synonymy with *T. cucumeris* (=*R. solani*). We included an isolate of *P. f. timsii* (CMI 61796) in this analysis, and it was identified as AG-1 IC (similarity indices ranging from 0.711–0.778).

Although the MIS was not able to differentiate all of the ISGs of *R. solani* AG-1, it has been used successfully to characterize and differentiate populations and ISGs of *R. solani* AG-2-2 and AG-3 (22,23). Although only 12 fatty acids have been detected in the isolates of *R. solani* studied thus far, qualitative and quantitative differences in fatty acid composition have allowed verification of isolate identity and differentiation of certain populations.

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