

Relationships and taxonomic status of *Alternaria radicina*, *A. carotiincultae*, and *A. petroselini* based upon morphological, biochemical, and molecular characteristics

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Abstract: *Alternaria radicina*, *A. carotiincultae*, and *A. petroselini* are closely related pathogens of umbelliferous crops. Relationships among these fungi were determined based on growth rate, spore morphology, cultural characteristics, toxin production, and host range. Random amplified polymorphic DNA (RAPD) analysis of these species, other species of *Alternaria*, and closely related fungi was also performed. *A. petroselini* was readily differentiated from *A. radicina* and *A. carotiincultae* on the basis of spore morphology, production of microsclerotia, host range, and RAPD analysis. *Alternaria radicina* and *A. carotiincultae* were considerably more similar to each other than to *A. petroselini*, but could be differentiated on the basis of growth rate, spore morphology, colony morphology, and, to a limited extent, RAPD analysis. When grown on media having a high nutritional content, *A. radicina* produced a diffusible yellow pigment and crystals of the fungal metabolite radicinin. In contrast, *A. carotiincultae* produced little or no radicinin. However, when *A. carotiincultae* was grown on the same medium amended with radicinin, growth rate and colony and conidial morphology were more similar to those of *A. radicina*. These results suggest that the morphological differences between *A. radicina* and *A. carotiincultae* are due, at least in part, to radicinin production, and that these fungi are conspecific. Therefore, we propose that *A. carotiincultae* be considered a synonym of *A. radicina*.

Key Words: Apiaceae, carrot black rot, radicinin, rDNA

INTRODUCTION

Alternaria radicina Meier, Drechsler and E. D. Eddy is the causal agent of carrot black rot disease, which causes carrot crop losses worldwide (CAB 1972,

Meier et al 1922). The fungus attacks all parts of the carrot plant including leaves, flowers, seeds, and roots. However, it is the necrotic black lesions induced in storage roots that result in the greatest economic losses (Grogan and Snyder 1952, Lauritzen 1926, Maude 1966). The host range of *A. radicina* is limited primarily to carrot. However, *A. radicina* also has been reported to cause a foliar blight of parsley and a stalk and root rot of celery (Tahvonon 1978, Wearing 1980).

Neergaard (1945) examined the morphological and cultural variation within the species *A. radicina* [as *Stemphylium radicinum* (Meier, Drechs. and E. D. Eddy) Neergaard 1939], and recognized three distinct types (a, b, and c) based on differences in colony morphology and, to a lesser extent, spore morphology and host range. Type a and b isolates were isolated from and were strongly pathogenic on carrot, whereas type c isolates were isolated from celery and were strongly pathogenic on celery and parsley. Neergaard (1945) further concluded that another closely related species, *A. petroselini* (Neergaard) Simmons (as *S. petroselini* Neergaard 1942), which is pathogenic on parsley, should be reclassified as *S. radicinum* var. *petroselini* Neergaard due to similarities in conidium morphology with *S. radicinum*.

The most significant differences between *A. radicina* type a and b isolates were in terms of their cultural characteristics (Neergaard 1945). On malt agar, type a isolates formed small colonies with irregular margins and produced tree-like crystals that grew into the medium. Type b isolates formed large colonies with even margins and usually did not produce crystals. The crystals produced by type a isolates were subsequently shown to be composed of radicinin, a keto-lactone secondary metabolite that has phytotoxic and antifungal properties (Aldridge and Grove 1964, Grove 1964, 1970). Radicinin also is produced by *Alternaria chrysanthami*, *Cochliobolus lunata*, *Bipolaris coicis*, and *Phoma andina* (Nakajima et al 1997, Noordeloos et al 1993, Nukina and Marumo 1977, Robeson et al 1982).

During the course of our research on carrot black rot disease in California, over 200 isolates of *A. radicina* were recovered from commercial carrot seed, carrot tissue, and soil. Most of these isolates (>98%) corresponded to Neergaard's type a, i.e., slow growth,

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irregular colony margin, and crystal production on potato dextrose agar (PDA). These isolates also produce a diffusible yellow pigment after 10–15 d on PDA or acidified PDA (APDA) (Pryor et al 1997). These isolates were designated as *A. radicina* type 1 by Pryor et al (1997). Six isolates, all recovered from carrot seed, had cultural characteristics that were more similar to those described by Neergaard as type b, i.e., rapid growth, even colony margin, and no crystal production. These latter isolates also did not produce a diffusible yellow pigment. These isolates were designated as *A. radicina* type 2 by Pryor et al (1997). Type 1 and 2 isolates were pathogenic on carrot (Pryor et al 1997).

In 1995, *A. carotiincultae* E. G. Simmons was described as a new species of *Alternaria* isolated from wild carrot from Ohio (Simmons 1995). Though similar in many respects to *A. radicina*, *A. carotiincultae* was classified as a distinct species based on having: (i) greater average conidium length, (ii) fewer obvoid and subspherical conidia, and (iii) a greater frequency of conidia produced in chains of two, or less commonly, three. A morphological comparison of the ex-type culture of *A. carotiincultae* with our *A. radicina* type 2 isolates suggested to us that they were conspecific.

In recent work, phylogenetic relationships among *Alternaria*, *Ulocladium*, and *Stemphylium* were determined based on analyses of nuclear 18S and ITS/5.8S, and mitochondrial small subunit (mt SSU) ribosomal DNA (rDNA) sequences (Pryor and Gilbertson 2000). This work revealed that *A. radicina* and *A. petroselini* were closely related, but had considerable divergence in 18S, ITS/5.8S, and mt SSU sequences. In contrast, *A. radicina* and *A. carotiincultae* had identical 18S and mt SSU sequences, and their ITS/5.8S sequences differed by one nucleotide. The high level of rDNA sequence identity between *A. radicina* and *A. carotiincultae* exceeds that found among *A. dauci*, *A. solani*, and *A. porri*, which have been considered as *formae speciales* of a single species by some authors (Joly 1964, Neergaard 1945). Because both *A. radicina* and *A. carotiincultae* cause similar disease symptoms in carrot and have nearly identical rDNA sequences, it is hypothesized that *A. radicina* and *A. carotiincultae* may be conspecific.

The purpose of this work was to further examine the relationship between *A. radicina* and *A. carotiincultae* in terms of morphological, biochemical, and molecular characteristics. Isolates of *A. petroselini* were included because of the close relationship of this species with *A. radicina*.

MATERIALS AND METHODS

Cultural and morphological characterization.—Three isolates each of *A. radicina*, *A. carotiincultae*, and *A. petroselini* were

TABLE I. Fungal isolates used in this study

Species	Source ^a	Designation
Isolates used in morphological, pathogenic, biochemical, and RAPD ^b analyses:		
<i>Alternaria carotiincultae</i>	BMP	21-21-13
	BMP	21-21-15
	BMP	21-21-16
<i>A. petroselini</i>	BMP	21-41-01
	BMP	21-41-02
	BMP	21-41-03
<i>A. radicina</i>	BMP	21-21-07
	BMP	21-21-11
	BMP	21-21-14
Atypical isolates:		
<i>A. radicina</i> (ex-neotype)	ATCC	6503
<i>A. radicina</i> (celery isolate)	ATCC	58405
Additional isolates used in RAPD analyses:		
<i>A. arborescens</i> ^c	ATCC	28329
<i>A. brassicicola</i>	EEB	2232
<i>A. carotiincultae</i> (ex-type)	EGS	26-010
<i>A. dauci</i>	ATCC	36613
<i>A. macrospora</i>	DGG	Amsl
<i>A. petroselini</i>	EGS	09-159
<i>A. porri</i>	ATCC	58175
<i>A. radicina</i> (representative isolate)	ATCC	96831
<i>A. solani</i>	ATCC	58177
<i>A. tenuissima</i>	ATCC	16423
<i>Stemphylium botryosum</i>	ATCC	42170
<i>S. callistephi</i>	EEB	1055
<i>S. sarcinaeforme</i>	EEB	1072
<i>S. vesicarium</i>	ATCC	18521

^a Abbreviations for sources are as follows: ATCC - American Type Culture Collection, Rockville, MD 20852; BMP - B. M. Pryor, Dept. of Plant Pathology, University of California, Davis, CA 95616; DGG - D. G. Gilchrist, Dept. of Plant Pathology, University of California, Davis, CA 95616; EEB - E. E. Butler, Dept. of Plant Pathology, University of California, Davis, CA 95616; EGS - E. G. Simmons, Crawfordsville, IN 47933.

^b Random Amplified Polymorphic DNA.

^c *A. alternata* f. sp. *lycopersici* (Simmons, E. G. 1999. Mycotaxon 70:325–369).

used to determine growth rate, conidium dimensions, and cultural characteristics of these species (TABLE I). *Alternaria radicina* and *A. carotiincultae* were isolated from infested carrot seed, and *A. petroselini* isolates were obtained from infested parsley seed. The species identification of these isolates was accomplished by comparison of morphological and cultural characteristics with those of ex-type or representative cultures for each species [*A. radicina*, ATCC (American Type Culture Collection) 96831; *A. carotiincultae*, EGS (E. G. Simmons) 26–010 (ex-type); and *A. petroselini*, EGS 09–159]. The species identity for most of these isolates was confirmed by E. G. Simmons (717 Thornwood Rd., Crawfordsville, Indiana 47933). Isolates BMP 21–21–07

and BMP 21–21–11 were identified as members of the radicina species-group, but were not confirmed as either *A. carotiincultae* or *A. radicina*. Isolates 21–41–01 and 21–41–02 were not examined by E. G. Simmons. All isolates were maintained on PDA (Difco, Plymouth, Minnesota) plates at 22 C, or on PDA slants at 10 C.

Fungal growth rates were determined on potato carrot agar (PCA) (Simmons 1992), V-8 juice agar (V8A) (Simmons 1992), PDA, and acidified PDA (APDA, pH 5.0). For each isolate, three mm agar plugs were removed from the margin of a 10-day-old culture on corn meal agar (CMA; Difco) and placed, mycelium side up, in the center of each of three 9-cm Petri dishes containing the appropriate medium. Petri dishes were incubated in clear plastic boxes with the surface of each dish 40 cm beneath fluorescent lights (Sylvania cool-white, 10/14 h light/dark) at 22 C. Colony diameters were measured after 8 d of incubation. Values for the three plates were averaged to obtain a mean radial growth rate for each isolate. This experiment was conducted twice. For each medium, Bartlett's test for non-homogeneity was used to evaluate homogeneity of variance within each experiment. A chi-square test for homogeneity of variance was used to determine if data from the separate trials could be combined for a single analysis. An analysis of variance (ANOVA) was performed to determine significant differences ($P \leq 0.05$) in growth rate among the three species for each medium.

Conidia from 10-day-old cultures on PCA, V8A, PDA, and APDA dishes, grown as described previously, were used to obtain conidium measurements. Conidia were taken from approximately 15 mm inside the colony margin in order to obtain uniform, mature conidia from an actively growing portion of the colony. Conidia were suspended in water and observed with a light microscope at 640 \times . Length and width measurements were taken from three categories of conidia: those having 3, 4, or 5 transepta. Irregular, subspherical conidia with oblique septa, as well as immature conidia (i.e., lacking pigmentation and longisepta) were not counted. Measurements were taken from ten randomly selected conidia for each category. Mean values and length/width (l/w) ratios were calculated. In addition, the number of transepta per conidium was determined for 50 randomly selected conidia in four fields of view (200 \times), and a mean number of septa per conidium were calculated. The maximum number of transepta per conidium was also noted for each species. For each medium, Bartlett's test for non-homogeneity was used to evaluate homogeneity of variance among isolates. ANOVA was performed to determine significant differences ($P \leq 0.05$) in conidium length, width, and l/w ratio for each conidium category, and in the number of transepta/conidium among the three species for each medium.

Cultural characteristics were noted for each isolate after 10–15 d of growth on PCA, V8A, PDA, and APDA dishes. The production of pigments, crystals, and microsclerotia were determined by visual examination of dishes. The production of conidia in catenate arrangement was determined by examining dishes using a dissecting microscope (100 \times) with substage illumination. The production of subspherical conidia was determined by examining a small section of

colony (approximately 1–2 mm²) mounted in water using a compound microscope (200 \times).

Radixinin production.—The capacity of each isolate to produce radixinin in liquid culture medium (20.7 g D-glucose, 1.2 g DL asparagine, 1.2 g K₂HPO₄·3H₂O, 0.5 g MgSO₄·7H₂O, 0.5 g yeast extract, 0.1 g NaCl, per liter of deionized H₂O) was determined. Inoculum for liquid cultures was prepared by flooding CMA plates containing 10-day-old cultures of each fungal isolate with 10 mL of sterile deionized H₂O, and dislodging most conidia and aerial mycelia with a plastic rod. For each isolate, two ml of this suspension was pipetted from the dish and added to 100 mL of liquid culture medium in a 500 mL Erlenmeyer flask. Flasks were placed on a rotary shaker at 120 rpm and incubated at 22 C for 10 d. After incubation, three mL of liquid medium were removed and extracted with chloroform as described by Robeson et al (1982). Radixinin was detected in chloroform extracts by thin-layer chromatography (TLC) analysis (Robeson et al 1982), and quantified by comparison with serial dilutions of reagent-grade radixinin (Sigma, St. Louis, Missouri) prepared in chloroform. This experiment was conducted three times.

The effect of radixinin on the growth of *A. radicina* and *A. carotiincultae* in culture was assessed by amending PDA with reagent-grade radixinin at concentrations of 100, 200, and 500 ppm. Three mm plugs with *A. radicina* (isolates 21–21–07 and ex-neotype ATCC 6503) or *A. carotiincultae* (ex-type EGS 26–010) were placed on dishes as previously described. After 10 d, the colony growth rate and average number of transepta per conidium was determined for each isolate on amended and non-amended media. This experiment was conducted three times. In addition, colony and conidium characteristics on amended and non-amended media were noted for each trial.

Pathogenicity tests.—The host range of the isolates was determined by inoculating 5-wk-old carrot, dill, fennel, cilantro, and parsley seedlings; 6-week-old anise, caraway, and parsnip seedlings; and 8-wk-old celery seedlings. All seedlings had 3–5 true leaves. Seeds were sown in 10.5 cm-diam plastic pots and seedlings were thinned to 5 plants per pot. A suspension of conidia was prepared for each of the 9 test isolates by flooding a V-8 agar dish containing a 10-day-old culture with 10 mL of sterile deionized H₂O and gently dislodging conidia with a plastic rod. Suspensions were filtered through two layers of cheesecloth, and the concentration of conidia was determined with a hemacytometer. Conidium suspensions were adjusted to 2000 conidia/mL, and sprayed onto leaves of each test plant, until run-off, with an aerosol spray bottle (Nalge Company, Rochester, New York). Two replicate pots of each host species were inoculated per isolate. One pot of uninoculated plants per host species was used as a control. Plastic bags were immediately placed over each pot, and secured at the base of each pot with rubber bands. After 48 h, the bags were removed and the pots were placed in a greenhouse mist chamber with periodic misting. Two wk after inoculation each plant was scored for the severity of disease with the following rating system: 0 = no disease, 1 = 1% leaf necrosis, 2 = 5% leaf necrosis, 3 = 10% leaf necrosis, 4 = 20% leaf necrosis, 5 = >40% leaf

necrosis. Disease ratings for plants within each pot were averaged to obtain a mean rating for each pot. The mean ratings of two replicate pots were averaged to obtain a mean pathogenicity score for each fungal isolate on each host. The pathogenicity tests were conducted three times. Bartlett's test for non-homogeneity was used to determine if there was homogeneity of variance within each trial. A chi-square test for homogeneity of variance among trials was performed to determine if the data from the three trials could be combined into a single analysis. ANOVA was performed to determine significant differences ($P \leq 0.05$) in the pathogenicity score among the three species for each host plant.

Molecular analysis.—RAPD analyses were performed with isolates of *A. radicina*, *A. carotiincultae*, and *A. petroselini* listed in TABLE 1, as well as 11 additional isolates representing the genera *Alternaria* and *Stemphylium* (TABLE 1). All isolates were maintained on PDA plates at 22 C or on PDA slants at 10 C. DNA was extracted from fungi grown in liquid culture as previously described (Pryor and Gilbertson 2000), and further purified with the Prep-a-gene DNA Purification System (Biorad Inc., Hercules, California). DNA concentrations were adjusted to 10 ng/ μ L for RAPD analyses.

RAPD analysis was performed using the 20 primers from Operon primer set A (Operon Technologies, Inc., Alameda, California). RAPD reactions were carried out in a 50 μ L reaction mixture (10 ng DNA, 0.5 mM each primer, 0.25 mM each dNTP, 2.5 mM $MgCl_2$, and 1.0 U Amplitaq DNA polymerase in 1 \times Amplitaq PCR buffer II [PE Applied Biosystems, Foster City, California]). PCR was carried out in a thermal cycler (Model 480, PE Applied Biosystems) programmed for the following parameters: 94 C—1 min, 34 C—1.5 min, 72 C—2 min for 45 cycles. PCR products were analyzed by electrophoresis in 1% agarose gels in TBE buffer (Sambrook et al 1989), and UV illumination after staining in ethidium bromide. RAPD reactions were conducted at least twice with each primer to confirm that RAPD patterns were reproducible.

The sizes of RAPD fragments were determined with an IS-1000 Digital Imaging System (Alpha Innotech Corp., San Leandro, California). All RAPD fragments between 500 and 2500 base pairs (bp) were scored with no correction for band intensity. Isolates were scored for the presence or absence of a given RAPD marker, and a binary matrix was constructed. Cluster analysis of the data matrix was performed by the Unweighted Pair-Group Method with Arithmetic mean (UPGMA) using the NTSYS-pc Numerical Taxonomy and Multivariate Analysis System software (version 1.80, Exeter Software, Setauket, NY). Results were graphically displayed in a dendrogram.

Analysis of atypical *A. radicina* isolates.—Two additional *A. radicina* isolates were examined during the course of this study: ATCC 6503, the ex-neotype of *A. radicina* (Simmons 1995), and ATCC 58405, an isolate from celery (Wearing 1980) (TABLE 1). Isolates were maintained on PDA plates at 22 C or on PDA slants at 10 C. Conidium measurements of each isolate were determined from cultures grown on PCA as previously described. Growth rate and cultural char-

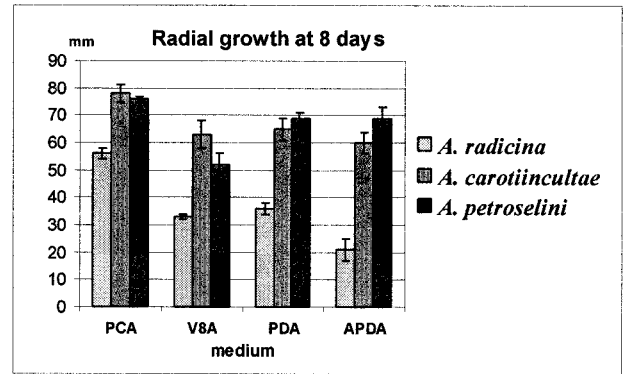


FIG. 1. Radial growth of *A. radicina*, *A. carotiincultae*, and *A. petroselini* on various media. For each fungus, values represent averages of 3 isolates for three independent experiments.

acteristics, including production of pigments, crystals, subspherical conidia, microsclerotia, and/or catenate conidia were determined on APDA plates as previously described. Isolate ATCC 6503 was examined for the production of radicinin in liquid culture as described previously. The pathogenicity of ATCC 6503 and ATCC 58405 on carrot, caraway, celery, cilantro, dill, fennel, parsley, parsnip, and anise seedlings was determined as previously described. RAPD analyses of each isolate were conducted as described previously. In addition, the nuclear ITS/5.8S and mt SSU rDNA sequences were determined and compared to sequences of type/representative cultures in GenBank. For the molecular analyses, growth of fungal tissue, extraction of fungal DNA, sequence analyses, and sequence comparisons were performed as previously described (Pryor and Gilbertson 2000).

RESULTS

Cultural and morphological characterization.—Statistical analysis revealed homogeneity of variance within and between the growth rate experiments for all 4 media tested. Thus, data from both experiments were combined into a single analysis. On all media, the growth of *A. radicina* was significantly less than that of *A. carotiincultae* or *A. petroselini* (FIG. 1), particularly on APDA where, in most cases, colony growth of the former ceased after 10–15 d. The growth of *A. carotiincultae* and *A. petroselini* was not significantly different on PCA or PDA, whereas on V8A the growth of *A. petroselini* was significantly less than that of *A. carotiincultae*, and on APDA the growth of *A. carotiincultae* was significantly less than that of *A. petroselini*.

Conidium measurements for the three species are presented in TABLE II. For each category of conidia, there was no significant difference in length, width, or l/w ratio of *A. radicina* and *A. carotiincultae* conidia. Conidia of *A. petroselini* were significantly wid-

TABLE II. Conidium measurements for *A. radicina*, *A. carotiincultae*, and *A. petroselini* grown on various media

Medium ^c	Species	Conidium measurements (l/ w/ 1/w ratio) ^a			# transverse septa/ conidium ^b	
		3-septa	4-septa	5-septa	Max	Avg
PCA	<i>A. radicina</i>	34a/ 16a/ 2.0b	41a/ 17a/ 2.4b	48a/ 18a/ 2.7b	7	4.0b
	<i>A. carotiincultae</i>	35a/ 17a/ 2.1b	44ab/ 18a/ 2.4b	50a/ 18a/ 2.8b	10	4.7c
	<i>A. petroselini</i>	37a/ 21b/ 1.8a	46b/ 22b/ 2.1a	54b/ 23b/ 2.3a	7	3.6a
V8A	<i>A. radicina</i>	38a/ 18a/ 2.1b	45a/ 19a/ 2.4a	50a/ 19a/ 2.6a	7	3.6a
	<i>A. carotiincultae</i>	35a/ 17a/ 2.1b	46a/ 18a/ 2.5a	53a/ 19a/ 2.8a	10	4.6b
	<i>A. petroselini</i>	39a/ 21b/ 1.9a	49a/ 22a/ 2.2a	54a/ 22a/ 2.5a	6	3.7a
PDA	<i>A. radicina</i>	37a/ 18a/ 2.1a	46a/ 19a/ 2.4b	52a/ 21a/ 2.5b	6	3.8a
	<i>A. carotiincultae</i>	38a/ 18a/ 2.1a	47ab/ 20a/ 2.4b	55a/ 21a/ 2.6b	9	4.5b
	<i>A. petroselini</i>	44b/ 22b/ 2.0a	50b/ 24b/ 2.1a	55a/ 24a/ 2.3a	6	3.6a
APDA	<i>A. radicina</i>	42a/ 19a/ 2.2a	47a/ 21a/ 2.2a	56ab/ 22b/ 2.5ab	6	3.3a
	<i>A. carotiincultae</i>	38a/ 18a/ 2.1a	46a/ 19a/ 2.4a	54a/ 19a/ 2.8b	8	3.9b
	<i>A. petroselini</i>	45a/ 23b/ 2.0a	53b/ 24b/ 2.2a	61b/ 25c/ 2.4a	5	3.1a

^a Measurements were obtained from conidia collected from approximately 15 mm inside of the colony margin of a 10-day-old culture. Suspensions of conidia were observed with a compound microscope at 640X, and measurements were taken from randomly selected conidia having 3, 4, or 5 transepta. For each conidium category, ten conidia were measured and a mean value was calculated. For each medium, values within each column followed by different letters are significantly different ($P \leq 0.05$).

^b Fifty conidia from each isolate were randomly observed in four fields of view (200X) and the number of transverse septa per conidium was counted and the mean number of septa per conidium was calculated. For each medium, values within each column followed by different letters are significantly different ($P \leq 0.05$).

^c Abbreviations for media are as follows: PCA, potato-carrot agar; V8A, V8 juice agar; PDA, potato-dextrose agar; APDA, acidified PDA.

er and, in most cases, longer compared with conidia of *A. radicina* and *A. carotiincultae*. In most cases, l/w ratios of *A. petroselini* conidia were significantly lower than those of *A. radicina* and *A. carotiincultae*.

Conidia of *A. carotiincultae* had significantly more transepta than those of *A. radicina* or *A. petroselini* (TABLE II). For example, on PCA *A. carotiincultae* conidia had a mean of 4.7 transepta, whereas *A. radicina* and *A. petroselini* conidia had means of 4.0 and 3.6 transepta, respectively. The maximum number of transepta per conidium was also consistently greater for *A. carotiincultae* compared with *A. radicina* or *A. petroselini* (TABLE II). The mean number of transepta per conidium for *A. petroselini* and *A. radicina* were not significantly different except on PCA.

Differences in colony morphology were observed, particularly on media with a high nutrient content (e.g., PDA and APDA) (TABLE III, FIG. 2). On such media, *A. radicina* colonies grew slowly and stopped growing after 10–15 d without covering the dish surface (FIG. 2). In addition, these colonies had irregular margins and produced dendritic crystals, characteristic of radicinin, and a yellow diffusible pigment (FIG. 3). In contrast, *A. carotiincultae* and *A. petroselini* colonies grew rapidly and covered the dish surface within 10–15 d, and these colonies had smooth margins (FIG. 2). *Alternaria carotiincultae* isolates did

not produce crystals or yellow pigments within 15 d (FIG. 2), although one isolate (21–21–13) produced very small crystals and a small amount of yellow pigment after 30 d of growth on APDA. *Alternaria petroselini* isolates produced few or no crystals, and trace amounts of yellow pigment on APDA.

All isolates of *A. carotiincultae* produced conidia in catenate arrangement on all media (TABLE III). *Alternaria radicina* isolates 21–21–07 and 21–21–11 commonly produced conidia in catenate arrangement on PCA, V8A, and PDA, but not on APDA. Production of conidia in catenate arrangement was uncommon for *A. radicina* isolate 21–21–14 on all media. The production of conidia in catenate arrangement by *A. petroselini* isolates was uncommon on PCA and V8A, and was not observed on PDA or APDA.

All isolates of *A. radicina* and *A. petroselini* produced subspherical conidia on all media (TABLE III). All isolates of *A. carotiincultae* commonly produced subspherical conidia on APDA but not on PCA, V8A, or PDA.

Alternaria petroselini isolates produced small microsclerotia (approximately 60–120 μm in diameter) (TABLE III, FIG. 4), which were distributed throughout the medium beneath the agar surface. Production of microsclerotia occurred on all media but was greatest on V8A. On V8A, microsclerotia were first

TABLE III. Cultural characteristics for *A. radicina*, *A. carotiincultae*, and *A. petroselini* grown on various media

Medium ^a	Characteristic	<i>A. radicina</i>	<i>A. carotiincultae</i>	<i>A. petroselini</i>
PCA	rapid growth ^b	+	+	+
	catenate conidia ^c	+	+	+/-
	subspherical conidia ^d	+	+/-	+
	pigment production ^e	-	-	-
	crystal production ^f	-	-	-
	microsclerotia ^g	-	-	+
V8A	rapid growth	+	+	+
	catenate conidia	+	+	+/-
	subspherical conidia	+	+/-	+
	pigment production	-	-	-
	crystal production	-	-	-
	microsclerotia	-	-	+
PDA	rapid growth	-	+	+
	catenate conidia	+	+	-
	subspherical conidia	+	+/-	+
	pigment production	+	-	+/-
	crystal production	+	-	-
	microsclerotia	-	-	+
APDA	rapid growth	-	+	+
	catenate conidia	+/-	+	-
	subspherical conidia	+	+	+
	pigment production	+	-	+/-
	crystal production	+	-	-
	microsclerotia	-	-	+

^a Abbreviations for media are as follow: PCA, potato-carrot agar; V8A, V8 juice agar; PDA, potato-dextrose agar; APDA, acidified PDA.

^b Rapid growth was recorded as (+) if the colony diameter was > 7 cm after 10 days, or (-) if colony diameter was < 6 cm after 10 days.

^c The presence of conidia in catenate arrangement was determined by examining dishes using a dissecting microscope (100X) and substage illumination, and was recorded as common (+) if several could be viewed in a single field of view, uncommon (+/-) if it was necessary to scan several fields of view to find one example, or (-) if absent.

^d The presence of subspherical conidia was determined by examining a small section of colony (approximately 1-2 mm²) mounted in water using a compound microscope (200X), and was recorded as common (+) if several such conidia could be viewed in a single field of view, uncommon (+/-) if it was necessary to scan several fields of view to find one example, or absent (-).

^e The production of pigment within 14 d was recorded as present (+), faint (+/-), or absent (-).

^f The production of crystals within 14 d was recorded as present (+) or absent (-).

^g The presence or absence of microsclerotia, formed within the agar medium after 14 d of growth, was recorded as present (+) or absent (-).

observed after 7 d of growth. Under the conditions of this study, *A. radicina* and *A. carotiincultae* did not produce microsclerotia.

Radicinin production.—All isolates grew well and similarly in liquid culture medium based upon visual comparisons of total fungal biomass produced during incubation. When grown in liquid culture, *A. radicina* isolates produced radicinin, and TLC analyses revealed that isolates produced 300–700 mg radicinin/L of medium. *Alternaria carotiincultae* isolates produced only trace amounts of radicinin (FIG. 5), consistent with the failure of these isolates to produce crystals in solid media. *A. petroselini* isolates produced more radicinin than *A. carotiincultae*, but consider-

ably less than *A. radicina*. Similar results were obtained in three independent experiments.

When *A. carotiincultae* was grown on PDA amended with radicinin (100 and 200 mg/l), colony growth after 10 d was less than that on non-amended medium, particularly at the 200 ppm rate (44 ± 0.9 mm versus 64 ± 0.7 mm, FIG. 6). Similarly, growth of *A. radicina* was reduced on PDA amended with radicinin compared with non-amended media, particularly at the 200 ppm rate (28 ± 2.1 versus 39 ± 2.2 mm, FIG. 6). Neither fungus grew on PDA amended with 500 ppm radicinin. In addition, when *A. carotiincultae* was grown on radicinin-amended media, the average number of transepta per conidium was re-

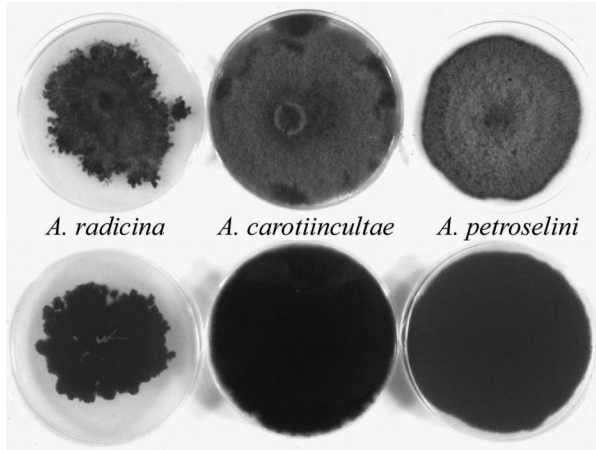


FIG. 2. Colony morphology of *A. radicina*, *A. carotiincultae*, and *A. petroselini* after 15 d of growth on acidified potato dextrose agar (top row shows upper surface of the Petri dish, bottom row shows the lower surface).

duced and more subspherical conidia were produced compared with conidia produced on non-amended media (e.g., 3.6 septa/conidium at 200 ppm radicinin versus 4.9 septa/conidium for non-amended medium; FIGS. 7A, B). Similar results were obtained in three independent experiments.

Pathogenicity tests.—Bartlett’s test revealed homogeneity of variance within each experiment for each host. However, the chi-square test revealed non-homogeneity of variance among the three experiments. Thus, the trials were analyzed separately.

A. radicina and *A. carotiincultae* were highly pathogenic on carrot seedlings (TABLE IV) and both fungi had similar disease ratings. Neither taxon was pathogenic on parsley seedlings, and both were weakly or moderately pathogenic on celery, cilantro, and fennel seedlings (TABLE IV). In contrast, *A. petroselini*

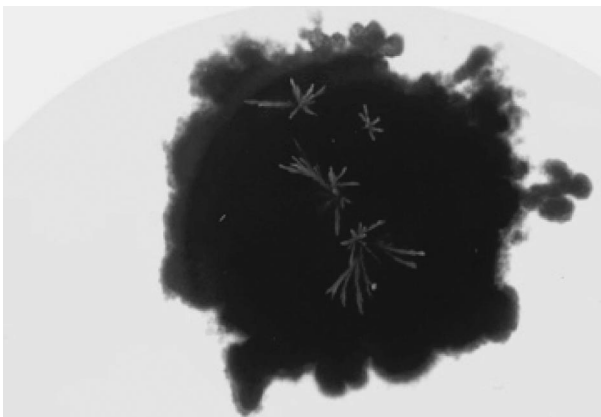


FIG. 3. Dendritic (tree-like) crystals of radicinin produced beneath a 15-day-old *A. radicina* colony growing on acidified potato dextrose agar.

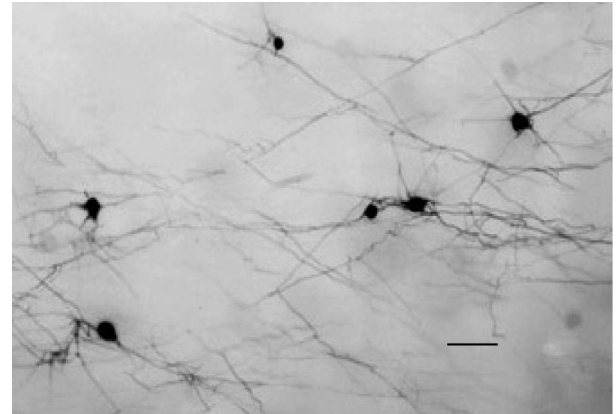


FIG. 4. Microsclerotia produced by *A. petroselini* after 15 days of growth on V-8 agar. Scale bar represents 0.9 mm.

was highly to moderately pathogenic on celery, cilantro, fennel, and parsley seedlings, but was not pathogenic on carrot (TABLE IV). All isolates of all taxa were weakly or not pathogenic on caraway, dill, parsnip and anise seedlings (data not shown). None of the control seedlings developed disease symptoms.

Molecular analysis.—Seventeen of the 20 random primers tested directed the amplification of reproducible RAPD fragments from total genomic DNA of all isolates tested, and a total of 710 markers were scored. Ten primers revealed polymorphisms between *A. radicina* and *A. carotiincultae*, whereas all 17 primers revealed polymorphisms between *A. radicina* and *A. petroselini* and between *A. carotiincultae* and *A. petroselini*. UPGMA cluster analysis of the RAPD data revealed 95% similarity between *A. radicina* and *A. carotiincultae*, a value considerably higher than those for the other fungal species examined

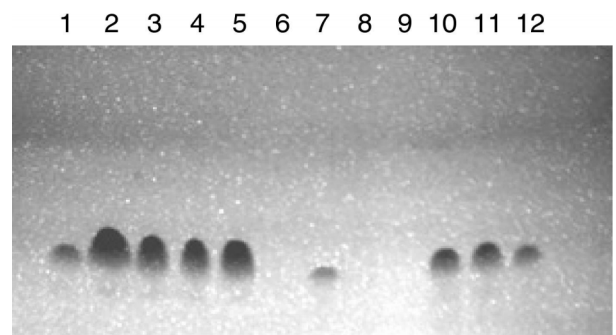


FIG. 5. Thin-layer chromatography analysis of chloroform extracts prepared from culture filtrates of 10-day-old liquid cultures of *A. radicina*, *A. carotiincultae*, and *A. petroselini*. Lanes 1 and 2 contain 100 and 500 ppm radicinin, respectively. Lanes 3–6, *A. radicina* isolates 21-21-07, 21-21-11, 21-21-14, and ATCC 6503. Lanes 7–9, *A. carotiincultae* isolates 21-21-13, 21-21-15, and 21-21-16. Lanes 10–12, *A. petroselini* isolates 21-41-01, 21-41-02, and 21-41-03.

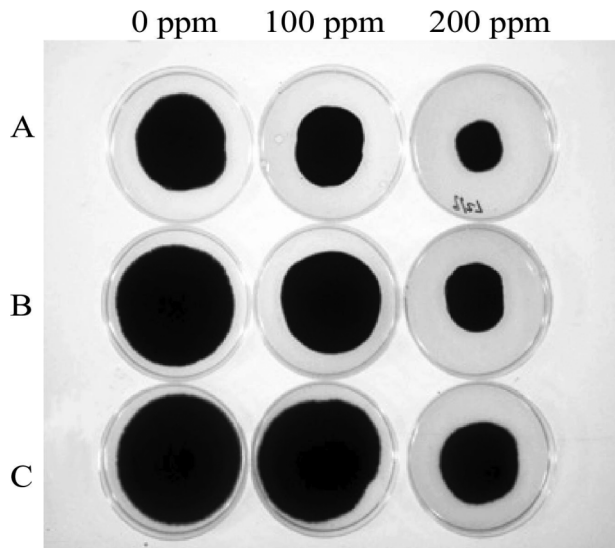


FIG. 6. Colony morphology of (A) *A. radicina* isolate BMP 21-21-07, (B) *A. carotiincultae* isolate BMP 21-21-15, and (C) *A. radicina* isolate ATCC 6503 after 10 days of growth on potato dextrose agar amended with 0, 100, or 200 ppm radicinin.

(FIG. 8). For example, the species with the next highest similarity values were *A. porri* and *A. solani* (88%). *A. petroselini* was more similar to *A. radicina* and *A. carotiincultae* (84%) than it was to other *Alternaria* species ($\leq 81\%$). RAPD analyses revealed few polymorphisms among the three *A. radicina* isolates, the three *A. carotiincultae* isolates, or the three *A. petroselini* isolates (FIG. 9).

Analysis of atypical A. radicina isolates.—Conidium measurements as well as maximum and average number of transepta per conidium for ATCC 6503 and ATCC 58405 are presented in TABLE V, and are compared with those of the other *A. radicina*, *A. carotiincultae*, and *A. petroselini* isolates used in this study (TABLE I). The sizes of conidia of ATCC 6503 were nearly identical to those of *A. radicina* (TABLE V). The sizes of conidia of ATCC 58405 were similar but not identical to those of *A. radicina*, whereas the l/w ratio and number of transepta were more similar to those of *A. petroselini* (TABLE V).

The growth of ATCC 6503 and ATCC 58405 on APDA after 8 d were 82 ± 0.8 cm and 78 ± 1.0 cm, respectively, which were considerably greater than those of *A. radicina* isolates and were comparable to those of *A. carotiincultae* and *A. petroselini* isolates, respectively (FIG. 1). On APDA, colony characteristics of ATCC 6503 included rapid growth and even margins; no production of crystals, pigments, or microsclerotia. These cultural characteristics are more similar to those of *A. carotiincultae* than those of *A. radicina* or *A. petroselini*. In addition, ATCC 6503 did

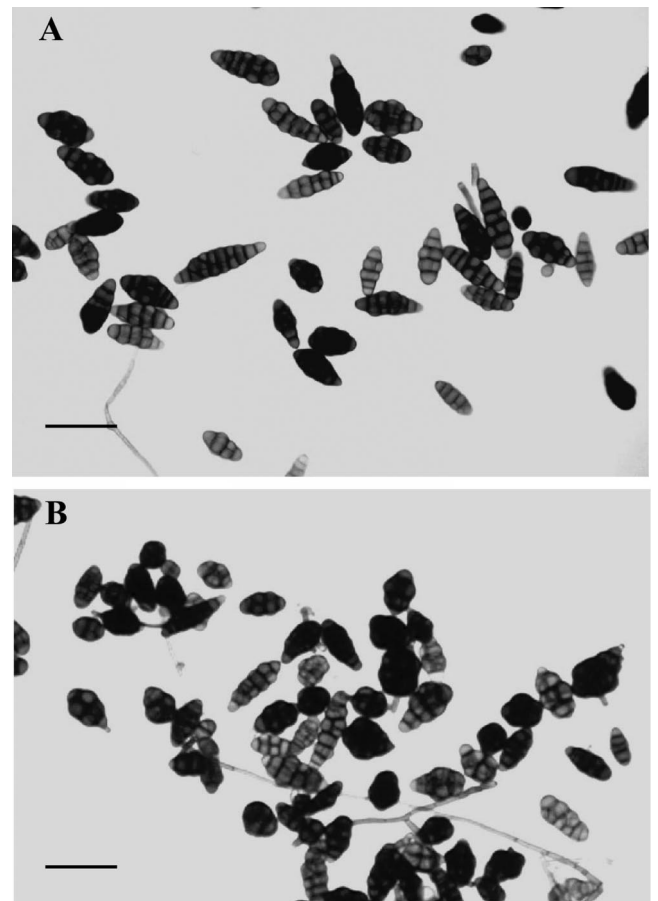


FIG. 7. Morphology of *A. carotiincultae* conidia after 10 days of growth on (A) PDA without radicinin and (B) PDA amended with 200 ppm radicinin. Scale bar represents 60 μ m.

not produce radicinin (FIG. 5). However, ATCC 6503 also produced few conidia in catenate arrangement, which is more similar to certain *A. radicina* isolates such as 21-21-14. On APDA, colony characteristics of ATCC 58405 included rapid growth and even margins, production of subspherical conidia and microsclerotia, but no production of crystals or catenate conidia and little production of yellow pigment. These cultural characteristics were more similar to those of *A. petroselini* than *A. radicina* or *A. carotiincultae*.

ATCC 6503 was pathogenic on carrot, weakly pathogenic on celery and cilantro, and non-pathogenic on parsley, dill, fennel, parsnip, caraway, and anise. In contrast, ATCC 58405 was highly pathogenic on celery, cilantro, fennel, and parsley, weakly pathogenic on caraway, carrot, and dill, and non-pathogenic on anise.

The RAPD patterns of ATCC 6503 were nearly identical to those of the *A. carotiincultae* isolates (FIG. 9). In contrast, the RAPD patterns of ATCC 58405

TABLE IV. Host range of *A. radicina*, *A. carotiincultae*, and *A. petroselini* based upon seedling pathogenicity tests with various Umbelliferous spp

Experiment	Species	Host ^a				
		Carrot	Celery	Cilantro	Fennel	Parsley
1	<i>A. radicina</i>	4.5b	1.5a	1.3a	2.3a	0.2a
	<i>A. carotiincultae</i>	4.8b	1.0a	1.3a	1.8a	0.2a
	<i>A. petroselini</i>	0.2a	4.3b	2.7b	4.0b	2.8b
2	<i>A. radicina</i>	2.3a	1.0*	0.8a	0.8a	0.2a
	<i>A. carotiincultae</i>	2.8a	1.3a	1.2a	0.7a	0.3a
	<i>A. petroselini</i>	0.0*	1.8b	2.0*	2.2b	2.2b
3	<i>A. radicina</i>	3.3b	1.8a	0.8a	1.2a	0.2a
	<i>A. carotiincultae</i>	4.8c	1.2a	0.2a	1.3a	0.3a
	<i>A. petroselini</i>	0.2a	2.7a	2.5b	3.2b	3.0b

^a Values represent the severity of disease based on the following scale: 0 = no disease, 1 = 1 % leaf necrosis, 2 = 5% leaf necrosis, 3 = 10% leaf necrosis, 4 = 20% leaf necrosis, 5 = >40% leaf necrosis. ANOVA was performed to determine significant differences among pathogenicity values. For each trial, values within each column followed by different letters were significantly different ($P \leq 0.05$). Asterisks (*) indicate data with no variance; statistical analysis was not performed.

were nearly identical to those of the *A. petroselini* isolates (FIG. 9).

The ITS4 and ITS5 primer pair directed the amplification of a 632 bp ITS/5.8S fragment from both ATCC 6503 and ATCC 58405, whereas the NMS1 and NMS2 primer pair directed the amplification of 668 and 699 bp mt SSU fragments from ATCC 6503 and ATCC 58405, respectively. All sequences were sub-

mitted to GenBank [ATCC 6503 accession numbers are AF307014 (ITS/5.8S) and AF307016 (mt SSU); ATCC 58405 accession numbers are AF307015 (ITS/5.8S) and AF307017 (mt SSU)]. Comparisons of the ATCC 6503 rDNA sequences with sequences from GenBank revealed 100% sequence identity with those of the *A. carotiincultae* ex-type culture (EGS 26-010). Comparisons of ATCC 58405 rDNA sequences with sequences from GenBank revealed 100% sequence identity with those of *A. petroselini* (EGS 09-159).

DISCUSSION

In this study, phenotypic and genotypic characters of *A. radicina*, *A. carotiincultae*, and *A. petroselini* were examined to more precisely determine the taxonomic relationships among these closely related fungal species. To date, the classification of these fungi as distinct species has been based primarily upon differences in conidium morphology (Simmons 1995). However, these morphological differences are relatively minor, making it difficult to differentiate these fungi, particularly if the plant host from which the fungus was isolated is not known. Moreover, the need to clearly define the taxonomy and nomenclature of these fungi is driven by economic and regulatory considerations. *Alternaria radicina* and *A. petroselini* are known seedborne pathogens of carrot and parsley, respectively, and their presence on commercial vegetable seed can result in rejection of seed lots by seed producers and growers. *Alternaria carotiincultae* has only recently been described (Simmons 1995), and non-experts in *Alternaria* taxonomy may fail to recognize it as distinct from *A. radicina*. Alternatively, in cases where *A. carotiincultae* is differentiated from

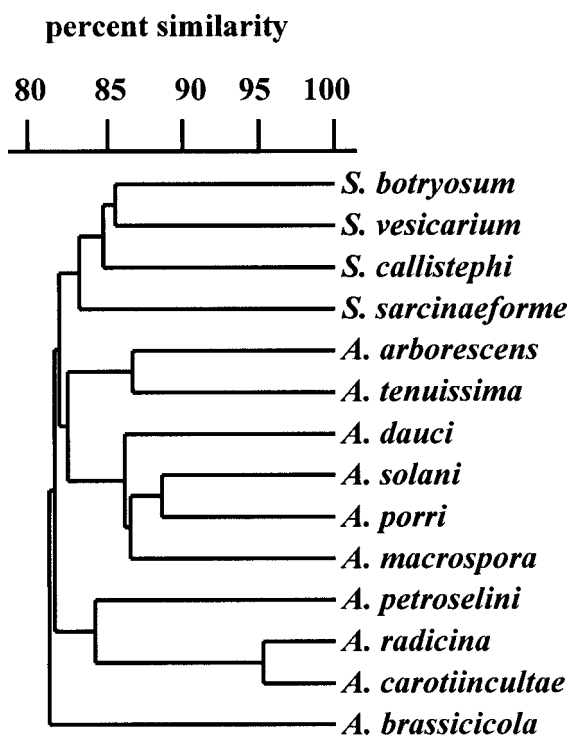


FIG. 8. Cladogram generated based on UPGMA cluster analysis of RAPD fragments from *Alternaria radicina*, *A. carotiincultae*, *A. petroselini*, and related fungi.

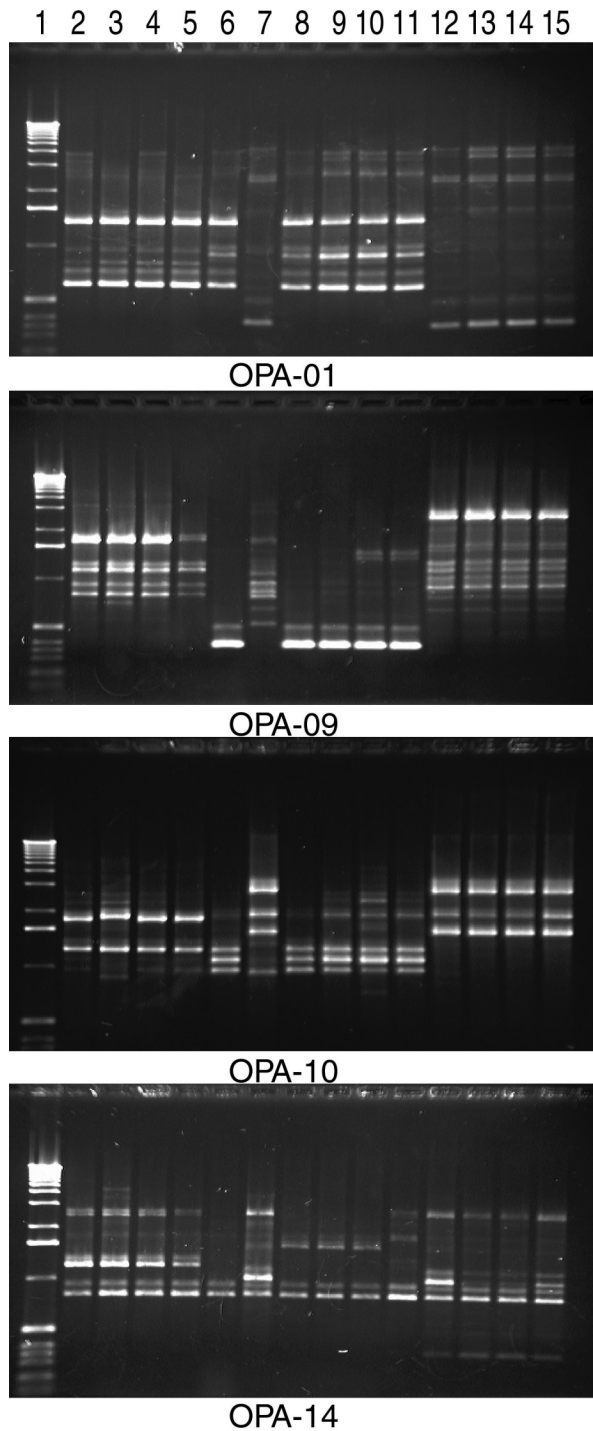


FIG. 9. Random amplified polymorphic DNA (RAPD) patterns generated from total genomic DNA of *A. radicina*, *A. carotiincultae*, and *A. petroselini* with four random primers from Operon Technologies (Alameda, CA). Lanes 2–5 are *A. radicina* isolates ATCC 96831 (representative isolate), BMP 21-21-07, BMP 21-21-11, and BMP 21-21-14, respectively; lanes 6 and 7 are *A. radicina* isolates ATCC 6503 (ex-neotype) and ATCC 58405 (celery isolate), respectively;

A. radicina, it may not be recognized as a carrot pathogen. As the economic importance of *A. carotiincultae* in black rot disease becomes more clear, carrot seed health regulations may need to be modified to address ecological, biological, and taxonomic differences between *A. carotiincultae* and *A. radicina*.

Several characteristics readily differentiated *A. petroselini* from *A. radicina* and *A. carotiincultae*. First, results of pathogenicity studies were consistent with previous studies establishing *A. petroselini* as a parsley pathogen and *A. radicina* and *A. carotiincultae* as carrot pathogens. Although *A. radicina* has been reported to be pathogenic on celery and parsley based on seedling assays conducted in test tubes (Neergaard 1945), neither *A. radicina* nor *A. carotiincultae* were highly pathogenic on celery or parsley in the seedling pathogenicity tests conducted in the present study. These differences are probably due to the different pathogenicity tests used or the age at which plants were inoculated or both. Nonetheless, *A. petroselini* can be readily differentiated from *A. radicina* and *A. carotiincultae* on the basis of pathogenicity on parsley and carrot seedlings.

Another distinguishing feature of *A. petroselini* was the production of microsclerotia in culture. Although cultural characteristics of *A. petroselini* have been determined in several previous studies, no microsclerotia were reported. Production of microsclerotia is not a common feature among *Alternaria* species, but has been reported for *A. japonica* (Tsuneda and Skoropad 1977). Thus, production of microsclerotia by *A. petroselini* may be a very useful characteristic for identification, particularly when the fungus is recovered from hosts other than parsley.

The usefulness of the above characteristics for identification of *A. petroselini* was demonstrated during the examination of the atypical *A. radicina* isolate, ATCC 58405, which was isolated from diseased celery. The conidium measurements of this isolate were reported to be similar to those of *A. radicina* (Wearing 1980) and this was confirmed in the present study. However, unlike *A. radicina*, this fungus grew rapidly on APDA, did not produce crystals in culture, and produced abundant microsclerotia on V8A within 7–10 d. Results of seedling pathogenicity tests revealed that this isolate was pathogenic on cel-

←

lanes 8–11 are *A. carotiincultae* isolates EGS 26-010 (ex-type), BMP 21-21-13, BMP 21-21-15, and BMP 21-21-16, respectively; lanes 12–15 are *A. petroselini* isolates EGS 09-159 (representative isolate), BMP 21-41-01, BMP 21-41-02, and BMP 21-41-03, respectively. Lane 1 contains 1 kb DNA ladder (Gibco BRL, Gaithersburg, MD).

TABLE V. Conidium measurements for atypical *A. radicina* isolates ATCC 6503 and ATCC 58405 and comparison with those of typical *A. radicina*, *A. carotiincultae*, and *A. petroselini* isolates

Isolate/Species	Conidium dimensions (l/ w/ l/w ratio) ^a			# transverse septa/conidium ^b	
	3-septa	4-septa	5-septa	Max	Avg
ATCC 6503	34/ 16/ 2.0	42/ 17/ 2.5	47/ 18/ 2.6	7	4.0
ATCC 58405	35/ 19/ 1.8	42/ 18/ 2.3	48/ 20/ 2.4	6	3.2
<i>A. radicina</i>	34/ 16/ 2.0	41/ 17/ 2.4	48/ 18/ 2.7	7	4.0
<i>A. carotiincultae</i>	35/ 17/ 2.1	44/ 18/ 2.4	50/ 18/ 2.8	10	4.7
<i>A. petroselini</i>	37/ 21/ 1.8	46/ 22/ 2.1	54/ 23/ 2.3	7	3.6

^a Measurements were obtained from conidia collected from approximately 15 mm inside of the colony margin of a 10-day-old culture grown on potato carrot agar. Suspensions of conidia were observed with a compound microscope at 640X, and measurements were taken from randomly selected conidia having 3, 4, or 5 transverse septa. For each conidium category, ten conidia were measured and a mean value calculated.

^b Fifty conidia from each isolate were randomly observed in four fields of view (200X) and the number of transepta per conidium was counted and the mean number of septa per conidium was calculated.

ery and parsley, but not on carrot. Furthermore, the RAPD patterns of ATCC 58405 and *A. petroselini* were nearly identical, and nuclear ITS/5.8S and mt SSU rDNA sequences of these isolates were identical. Thus, although the conidia of ATCC 58405 are morphologically similar to *A. radicina*, all other characteristics suggest that it is an atypical isolate of *A. petroselini*. These results further highlight the difficulties associated with identification of these fungi.

Alternaria radicina and *A. carotiincultae* are considerably more difficult to differentiate from each other than from *A. petroselini*. *Alternaria radicina* and *A. carotiincultae* were both pathogenic on carrot seedlings and neither fungus produced microsclerotia in culture. Furthermore, both fungi produced conidia that were morphologically similar, particularly if comparisons were made between conidia with the same number of transepta. *Alternaria radicina* could be differentiated from *A. carotiincultae* on the basis of the average number of transverse septa per conidium and maximum conidium length. However, this involved extensive comparative studies conducted under specifically defined culture conditions, which suggests that these characteristics may not be discerned during routine seed assays.

A key morphological feature used to differentiate *A. carotiincultae* from *A. radicina* is the production of conidia in a catenate arrangement (Simmons 1995). However, in the present study, the production of conidia in chains was not a reliable characteristic for differentiating these fungi. All *A. carotiincultae* isolates produced some conidia (approximately 10%) in catenate arrangement, whereas some *A. radicina* isolates did (e.g., isolates 21-21-07 and 21-21-11) and others did not (e.g., isolate 21-21-14). In the original description of *A. radicina*, conidia were described as produced "directly on sporophores or more tardily in catenulate arrangement, or singly at the apex of

short sporophoric processes arising from a spore of the preceding order as attenuated prolongation of terminal segment, or as lateral outgrowth of nonterminal, most frequently of basal segment" (Meier et al 1922). This statement suggests that *A. radicina* has the capacity to produce conidia in chains, but that it may be delayed or is variable, which is consistent with our findings.

Alternaria radicina and *A. carotiincultae* could be differentiated on the basis of cultural characteristics on APDA. *Alternaria radicina* isolates grew slowly and produced dendritic crystals and a diffusible yellow pigment, characteristics associated with the production of radicinin (Grove 1964, Noordeloos et al 1993). In contrast, *A. carotiincultae* grew more rapidly and did not produce crystals or pigment. TLC analysis confirmed that *A. radicina* isolates produced high levels of radicinin and that *A. carotiincultae* isolates produced little or no radicinin. These results suggested a correlation between the morphology of these fungi and the production of radicinin. Definitive evidence for this association came from studies in which the morphological properties of *A. carotiincultae* were altered when grown on media amended with radicinin. Radicinin inhibited the growth of *A. carotiincultae*, resulting in a reduced colony size and production of conidia that were shorter, had fewer longisepta, and more obovoid to subspherical in shape. Significantly, these are some of the key morphological characteristics used to differentiate *A. radicina* from *A. carotiincultae*. Thus, production of radicinin may be responsible, in part, for the morphological differences between *A. radicina* and *A. carotiincultae*. Further work on the effects of mycotoxins on fungal morphology may lead to new insight into taxonomic relations among other species of *Alternaria* and other fungi that produce secondary metabolites.

RAPD analysis provided a method to distinguish among *A. radicina*, *A. carotiincultae*, and *A. petroselini* that was not influenced by cultural and/or environmental conditions. Based on these analyses, *A. petroselini* was clearly differentiated from *A. radicina*, *A. carotiincultae*, and other *Alternaria* spp., consistent with its being a distinct species. However, the RAPD analysis revealed a higher level of genetic similarity between *A. radicina* and *A. carotiincultae* than between any of the other fungi examined. This included members of the *A. porri* species-group, some of which have been suggested to be *formae speciales* rather than distinct species (Joly 1964, Neergaard 1945). The genetic similarities between *A. radicina* and *A. carotiincultae* revealed by the RAPD analysis also were in agreement with previous rDNA sequence analyses showing that *A. radicina* and *A. carotiincultae* have identical nuclear 18S and mt SSU rDNA sequences, and nearly identical (one nucleotide difference) nuclear ITS/5.8S rDNA sequences (Pryor and Gilbertson 2000). Together, these results suggest a very close phylogenetic relationship between *A. radicina* and *A. carotiincultae*.

The difficulties in differentiating *A. radicina* and *A. carotiincultae* isolates were further demonstrated in the examination of the *A. radicina* ex-neotype, ATCC 6503. This isolate originated from J. I. Lauritzen and is presumably the isolate from which the type drawing of *A. radicina* by C. Drechsler was based (Meier et al 1922, Simmons 1995). Conidium measurements determined in this study were consistent with those presented in the original description and by Simmons (1995). However, the cultural characteristics of this isolate are atypical of *A. radicina*. The fungus grew rapidly, had even colony margins, and did not produce crystals or yellow pigments. TLC analysis confirmed that this isolate did not produce radicinin. Furthermore, in contrast to most *A. radicina* isolates, ATCC 6503 sporulated very poorly, a feature noted by Simmons and by Drechsler in earlier work (Simmons 1995). In the protologue of *A. radicina*, Meier et al (1922) stated "The fungus developed rapidly on potato agar, forming a dense, black mycelial growth over the surface, and after a few days produced conidiophores and conidia in great numbers". In addition, these authors stated "It may be mentioned that when the fungus is cultivated on substrata encouraging a rapid and abundant development, growth comes to a standstill comparatively early, stopping not unusually within 10 to 15 d". The authors further state that "Such cessation of growth is, of course, familiar enough to students of nearly every group of fungi, and finds some plausible explanation in the accumulation of toxins". Thus, in a number of characteristics, ATCC 6503 differs from

the original description of *A. radicina* (Meier et al 1922) and from field isolates of *A. radicina* (e.g., slow growth, abundant sporulation, and production of crystals and yellow pigment). In recognition of the differences between ATCC 6503 and most *A. radicina* isolates, an additional isolate was submitted to ATCC (ATCC 96831) as more representative of the species (Simmons 1995).

Thus, based on cultural, morphological, and molecular characteristics, ATCC 6503 is clearly an isolate of *A. carotiincultae*. If ATCC 6503 (*A. carotiincultae*) was derived from the original work of Meier et al (1922), it appears that these authors did not differentiate it from *A. radicina*. On the other hand, Neergaard (1945) may have differentiated these fungi based upon cultural characteristics as his descriptions of *A. radicina* types a and b are comparable to those of *A. radicina* and *A. carotiincultae*, respectively. Additionally, Neergaard's *A. radicina* type c, isolated from celery, may represent an atypical *A. petroselini* such as ATCC 58405. These previous taxonomic studies demonstrate the difficulty in differentiating isolates of *A. carotiincultae* and *A. radicina*.

Our examination of the relationships among these fungi considered morphological, cultural, ecological, biochemical, and molecular characteristics. Together, the results indicate a very close relationship exists between *A. radicina* and *A. carotiincultae*. Thus, these fungi would appear to represent either the minimal divergence for classification as distinct species or variants of a single species. Because *A. radicina* and *A. carotiincultae* are carrot pathogens that cause similar disease symptoms and some of the morphological differences between these fungi may be explained in terms of toxin production, we propose that they are conspecific and should be considered as variants of *A. radicina* rather than distinct species. Therefore, we propose that *A. carotiincultae* be considered a synonym of *A. radicina*.

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LITERATURE CITED

- Aldridge DC, Grove JF. 1964. Metabolic products of *Stemphylium radicinum*. Part II. (-)-7-hydroxy-4-oxo-oct-2-en-6-ynoic acid lactone. J Chem Soc 3239-3241.
- Commonwealth Agricultural Bureau (CAB). 1972. CMI index descriptions of pathogenic fungi and bacteria No. 346. Commonwealth Mycol. Inst., Kew.

- Grogan RG, Snyder WC. 1952. The occurrence and pathological effects of *Stemphylium radicinum* on carrots in California. *Phytopathology* 42:215–218.
- Grove JF. 1964. Metabolic products of *Stemphylium radicinum*. Part I. Radicinin. *J Chem Soc* 3234.
- . 1970. Metabolic products of *Stemphylium radicinum*. Part III. Biosynthesis of radicinin and pyrenophorin. *J Chem Soc (C)* 1860.
- Joly P. 1964. Le Genre *Alternaria*. *Encycl Mycol* 33:1–250.
- Lauritzen JI. 1926. The relation of black rot to the storage of carrots. *J Agric Res* 33:1025–1041.
- Maude RB. 1966. Studies on the etiology of black rot, *Stemphylium radicinum* (Meier, Drechsl., & Eddy) Neerg., and leaf blight, *Alternaria dauci* (Kuhn) Groves & Skolko, on carrot crops; and on fungicide control of their seed-borne infection phases. *Ann Appl Biol* 57:83–93.
- Meier FC, Drechsler C, Eddy ED. 1922. Black rot of carrots caused by *Alternaria radicina* N. Sp. *Phytopathology* 12:157–168.
- Nakajima H, Ishida T, Otsuda Y, Hamasaki T, Ichinoe M. 1997. Phytotoxins and related metabolites produced by *Bipolaris coicis*, the pathogen of Job's Tears. *Phytochemistry* 45:41–45.
- Neergaard P. 1945. Danish Species of *Alternaria* and *Stemphylium*. London: Oxford University Press. 560 p.
- Noordeloos ME, Gruyter J De, Eijl GW Van, Roeijmans HJ. 1993. Production of dendritic crystals in pure cultures of *Phoma* and *Ascochyta* and its value as a taxonomic character relative to morphology, pathology and cultural characteristics. *Mycol Res* 97:1343–1350.
- Nukina M, Marumo S. 1977. Radicinol, a new metabolite of *Cochliobolus lunata*, and absolute stereochemistry of radicinin. *Tetrahedron Letters* 37:3271–3272.
- Pryor BM, Davis RM, Gilbertson RL. 1997. Morphological and molecular characterization of *Alternaria radicina*, a review of the species. *Inoculum* 48:3. (Abstract)
- Pryor BM, Gilbertson RL. 2000. Molecular phylogenetic relationships amongst *Alternaria* species and related fungi based upon analysis of nuclear ITS and mt SSU rDNA sequences. *Mycol Res* 104:1312–1321.
- Robeson DJ, Gray GR, Strobel GA. 1982. Production of the phytotoxins radicinin and radicinol by *Alternaria chrysanthemi*. *Phytochemistry* 21:2359–2362.
- Sambrook J, Fritsch EF, Maniatis T. 1989. Molecular cloning: a laboratory manual (3). Cold Spring Harbor: Cold Spring Harbor Laboratory Press. 316 p.
- Simmons EG. 1992. *Alternaria* taxonomy: current status, viewpoint, challenge. In: Chelkowski J, Visconti A, eds. *Alternaria* Biology, Plant Diseases and Metabolites. Amsterdam: Elsevier Science Publishers BV. 573 p.
- . 1995. *Alternaria* themes and variations (122–144). *Mycotaxon* 55:55–163.
- Tahvonen R. 1978. Seed-borne fungi on parsley and carrot; *Alternaria dauci*, *Stemphylium radicinum*, *Septoria petroselinii*. *J Sci Agric Soc Finl* 50:91–102.
- Tsuneda A, Skoropad WP. 1977. Formation of microsclerotia and chlamydospores from conidia of *Alternaria brassicae*. *Can J Bot* 55:1276–1281.
- Wearing AH. 1980. *Alternaria radicina* on celery in South Australia. *APP Aust Plant Pathol Melbourne: Australian Plant Pathology Society* 9:116.