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Characterization of a Basidiomycete fungus from stored sugar beet roots

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Abstract: Eighteen isolates from sugar beet roots associated with an unknown etiology were characterized based on observations of morphological characters, hyphal growth at 4–28 C, production of phenol oxidases and sequence analysis of internal transcribed spacer (ITS) and large subunit (LSU) regions of the ribosomal DNA (rDNA). The isolates did not produce asexual or sexual spores, had binucleate hyphal cells with clamp connections, grew 4–22 C with estimated optimal growth at 14.5 C and formed a dark brown pigment on potato dextrose or malt extract agar amended with 0.5% tannic acid. Color changes observed when solutions of gum guiac, guiacol and syringaldzine were applied directly to mycelium grown on these media indicated that all isolates produced phenol oxidases. Sequences of ITS and LSU regions on the rDNA gene from 15 isolates were 99.2–100% identical, and analysis of sequence data with maximum likelihood and maximum parsimony suggest that the isolates from sugar beet roots are phylogenetically related to *Athelia bombacina*, *Granulobasidium vellereum* and *Cyphella digitalis*. High statistical support for both loci under different criteria confirmed that *Athelia bombacina* was consistently the closest known relative to the sugar beet isolates. Additional taxonomic investigations are needed before species can be clarified and designated for these isolates.

Key words: Agaricomycotina, Basidiomycota, beet storage, *Beta vulgaris*, root rot

Introduction

A fungus of unknown etiology exhibiting white, cottony mycelial growth was observed on roots of sugar beet (*Beta vulgaris* L.) harvested from commercial fields in 2006 and 2007 in Idaho (USA) after approximately 60 d at 1.7 C under high relative humidity (97–100%) indoors (Fig. 1A, B). Fungal growth continued after the initial observation, and mycelium extended 15 cm or more from the sugar beet roots after 90 d and formed a white crust on the surface of the roots when removed from humid environment. Similar observations were made on roots of sugar beet stored in outdoor piles under ambient environmental conditions.

The presence of the unknown fungus was shown by Strausbaugh et al. (2009) to be correlated with loss of sucrose from stored sugar beet roots, particularly from roots infected with Beet necrotic yellow vein virus (BNYVV). For example, when sugar beet roots were infected with BNYVV and stored in an indoor facility in Paul, Idaho, in 2007 and 2008, 27 and 40% of the root surface was covered with growth of the unknown fungus respectively and the roots lost 90–100% of their sucrose (Strausbaugh et al. 2009). Similar results also were observed on BNYVV-infected sugar beet roots colonized with the fungus when stored in outdoor piles (Strausbaugh et al. 2009). Although the precise role that the virus plays in sucrose loss by the fungus in long-term storage remains to be determined, the authors hypothesized that compromised root health due to virus infection before storage and increased respiration of sugar beet roots during storage might have contributed to the increased loss of sucrose. These findings are of concern to the sugar beet industry given that approximately two-thirds of the Idaho sugar beet crop is stored either indoors or outdoors before processing (Peterson et al. 1984).

Initial isolations performed from areas of fungal growth on sugar beet roots sampled from indoor and outdoor storage environments revealed mycelia with clamp connections, suggesting that the unknown fungus belonged to the phylum Basidiomycota (Strausbaugh et al. 2009). The objective of this study was to further characterize the fungus associated with stored sugar beet roots based on morphological and physiological characters and sequence analysis of ribosomal DNA.

Materials and methods

Isolates.—Eighteen isolates from sugar beet in Idaho were examined (Table I). Isolates were stored in potato dextrose broth with 50% glycerol at −80 C.

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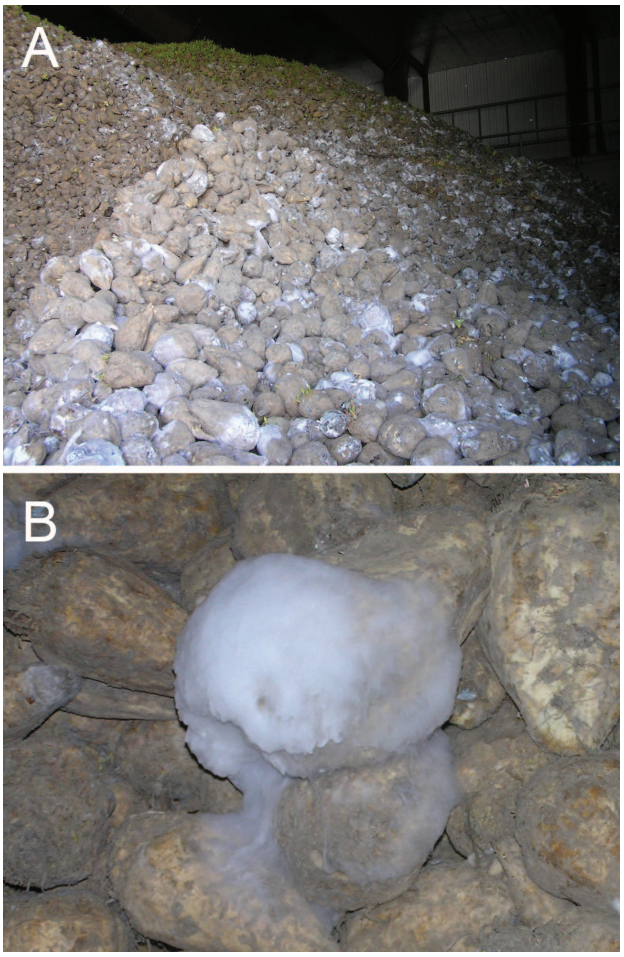

FIG. 1. Fungal colonization of sugar beet roots stored indoors in Paul, Idaho.

Hyphal growth and morphology.—Isolates were transferred to a 9 cm plastic Petri dish containing 20 mL potato dextrose agar (PDA; Becton Dickinson & Co., Sparks, Maryland) for 7 d at 24 C in the dark. Mycelial plugs (5 mm diam) taken from the advancing margin of a pure culture of each isolate were transferred to two replicate 9 cm Petri dishes containing 20 mL either potato dextrose (PDA), malt extracted (MEA), yeast extract soluble starch (YPSS) or 2% water agar (WA). Plates were incubated at 24 C in the dark, and radial growth was measured after 1, 3, 7 and 14 d. Colony morphology of each isolate was recorded for each medium until 30 d. Seven-day-old cultures of each isolate grown on PDA were transferred onto PDA plates covered by sterilized cellophane membrane and incubated at 24 C in the dark 5 d. After incubation the cellophane membrane with mycelia was cut into 2 × 10 mm^2 sections with a sterile scalpel, transferred onto a glass slide and stained by adding one drop each of 0.6% phenosafranin (Sigma) and 3% KOH. The slides were examined microscopically at 400× magnification to determine hyphal width, number of nuclei per cell in 10 cells and for the presence or absence of clamp connections. There were two replicates of each treatment, and the experiment was conducted twice.

TABLE I. Source of isolates obtained from sugar beet roots stored in an indoor storage facility in Paul, Idaho, (USA) with a temperature set point of 2 C

Culture number	Location	Cultivar[a]
F566	Near Paul, Idaho	C-17
F567	Near Paul, Idaho	B-30
F568	Near Paul, Idaho	HM070005
F569	Near Paul, Idaho	HH001
F570	Near Paul, Idaho	B-30
F571	Near Nampa, Idaho	SX001
F572	Near Nampa, Idaho	SX006
F573	Near Nampa, Idaho	HM070007
F574	Near Nampa, Idaho	HM070004
F575	Near Nampa, Idaho	B-16
F576	Near Nampa, Idaho	HM070015
F577	Near Nampa, Idaho	HH005
F578	Near Nampa, Idaho	HH001
F579	Near Nampa, Idaho	HH003
F580	Idaho	Unknown
F581	Idaho	Unknown
F582	Idaho	Unknown
F583	Idaho	Unknown

[a] For information on coded cultivars contact the respective companies: B = Betaseed Inc., C = ACH Seeds Inc., HH = Holly Hybrids, HM = Hilleshog and SX = Seedex.

Production of phenol oxidases.—Isolates were tested for production of phenol oxidase enzymes by growing them 14 d on MEA or PDA amended with 0.5% tannic acid (Sigma) at 24 C. Plates were examined for the presence of a dark brown pigmented region in the medium on the upper and underside of the fungal colony. A 30 µL drop of either guiacol (10% in 95% EtOH, Sigma), gum guiac (10% in 95% EtOH, Sigma) or syringaldezine (1% in 70% EtOH) solution was applied to the advancing edge of the colony and observed 30 min at 5 min intervals for color changes that indicated enzyme activity. The enzyme solutions (30 µL) were added to fungal mycelium grown on a plate of PDA or MEA without 0.5% tannic acid served as the controls. Each treatment was replicated three times, and the experiment was conducted twice.

Hyphal growth temperature experiments.—All 18 isolates (TABLE I) were grown on PDA in the dark by placing a 6 mm agar plug fungus side down in the center of the 9 cm Petri dish. The plates were arranged in a randomized complete block design with four replicates and incubated at 4, 10, 16, 22 and 28 C for 3 wk. Growth from the edge of the plug to the leading edge of the fungal growth was recorded weekly. The experiment was repeated once. Data were analyzed in SAS (9.2, SAS Institute Inc., Cary, North Carolina) with the general linear models procedure (Proc GLM) and regression (Proc reg). Fisher's protected least significant difference was used for mean comparisons. Bartlett's test was used to determine the homogeneity of variance.

DNA extraction.—All isolates were grown on PDA and transferred onto PDA covered with a sterilized cellophane membrane and incubated 10 d at 24 C in the dark. Mycelia were ground with a sterilized mortar and pestle with liquid nitrogen. Twenty to forty milligrams powdered mycelium was used for DNA extraction with the QIAGEN DNeasy kit (QIAGEN Science, Germantown, Maryland) following the manufacturer's instructions. The concentration of extracted DNA was measured by nanodropspectrophotometer (ND-1000, NanoDropTech-nologies, Wilmington, Delaware), diluted to 50 ng/μL, and stored at −20 C until further use with the polymerase chain reaction (PCR).

Polymerase chain reaction (PCR).—Genomic DNA of each isolate was used for PCR amplification of the internal transcribed spacer (ITS) and a portion of the large subunit (LSU) regions of the ribosomal DNA (rDNA) repeat with the primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′); and LR0R (5′-ACCCGCTGAACTTAAGC-3′) and LR7 (5′-TACTACCAC-CAAGATCT-3′) respectively (White et al. 1990, http://www.biology.duke.edu/fungi/mycolab/primers.htm). PCR amplification of the ITS and LSU regions was conducted with 50 ng DNA obtained from each isolate. The 50 μL reaction mixture consisted of 200 μM each dATP, dCTP, dGTP and dTTP, 50 pmol oligonucleotide primers, 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM MgCl and 1.25 units *Taq* DNA polymerase (Bioline USA Inc., Taunton, Massachusetts). The DNA Thermal Cycler (Mastercycler ep; Eppendorf, Hamburg, Germany) was programmed for 1 cycle of 5 min at 94 C; followed by 25 cycles of 30 s at 94 C, 30 s at 59 C, and 1 min at 72 C; and 1 cycle of 7 min at 72 C. The amplified products were observed by electrophoresis on 1% agarose gel (Agarose low EEO: Fisher Bioreagents, New Jersey) with Tris-acetic EDTA (TAE) buffer. The agarose gels were stained with ethidium bromide and visualized under UV transillumination. Amplified PCR products were purified with QIAquick PCR kit (QIAGEN). Purified products were sequenced with Big Dye Terminator Chemistry (3.1, Applied Biosystems) and submitted to the Duke IGSP Sequencing and Genetic Analysis Facility in Durham, North Carolina. Forward and reverse chromatograms were aligned and adjusted by visual examination with Sequencher™ (Gene Codes Corp., Ann Arbor, Michigan). Sequences were submitted to GenBank (accession Nos. AB596010–AB596041). Sequences were compared with those in GenBank with BLASTn 2.2.17 (Altschul et al. 1997) and GENETYX-MAC 12.0.1 (GENETYX Corp., Tokyo, Japan)

Analysis of sequence data.—Forward and reverse chromatograms were aligned and sequence ambiguities were resolved by visual examination with Sequencher. The sequences were aligned with Clustal W 1.7 (Thompson et al. 1994). The resulting alignment files were imported into MacCLade 4.08 (Maddison and Maddision 1997) for manual editing and exclusion of all ambiguously aligned regions. Heuristic searches for maximum parsimony (MP) and maximum likelihood were conducted in PAUP* (Swofford 1999). Model parameter estimates for ML analysis were obtained with Modeltest (Posada and Crandall 1998). Statistical support was calculated from 1000 bootstrap replicates.

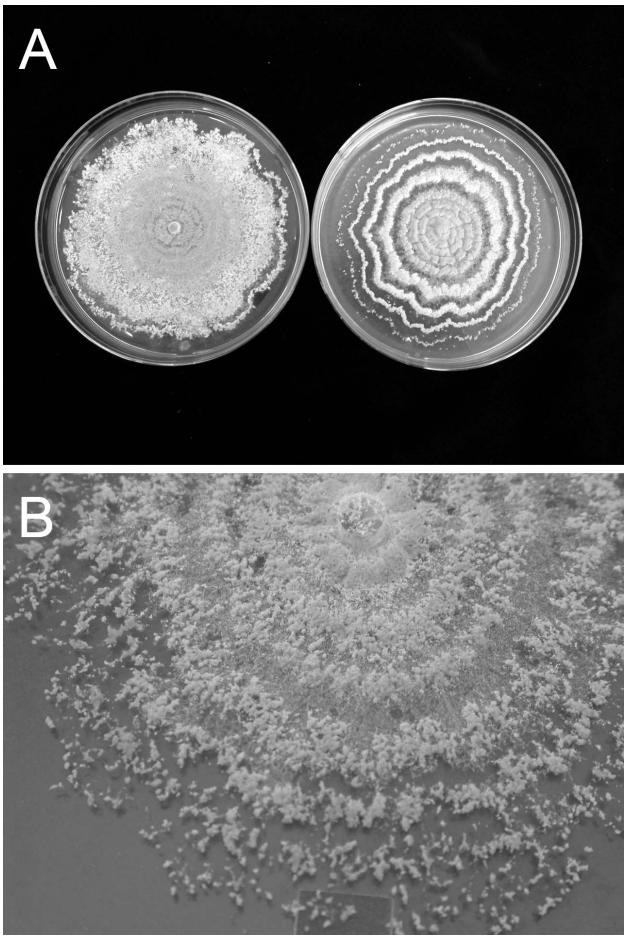

FIG. 2. Pure cultures of sugar beet isolate F567 on malt extract (left) and potato dextrose agar (right) after 28 d incubation at 24 C (A). Orange, sclerotial-like structures were formed after 6–10 d incubation (A, B).

RESULTS

Hyphal growth and norphology.—All isolates produced white mycelium on PDA and MEA. The isolates also produced sclerotial-like structures after 6 d incubation (FIG. 2A, B). These structures were initially white and turned light orange-brown after 10 d incubation and usually formed in concentric rings every 4–10 mm (FIG. 2A, B). Isolates grown on YPSS and WA produced whitish mycelium (data not shown) with a mean radial growth rate of 0.83 ± 0.7, 0.76 ± 0.8 (SD = 1.04 for WA, SD = 0.99 for YPSS) mm per day at 24 C. The isolates produced white sclerotial-like structures that turned brown after 14–16 d incubation but did not form these structures in a zonate pattern. Microscopic examination of the sclerotial-like structures showed aggregates of swollen hyphal cells that were not differentiated into a rind, cortex or medulla (FIG. 3A). All isolates had septate mycelium with clamp connections, two nuclei per hyphal cell

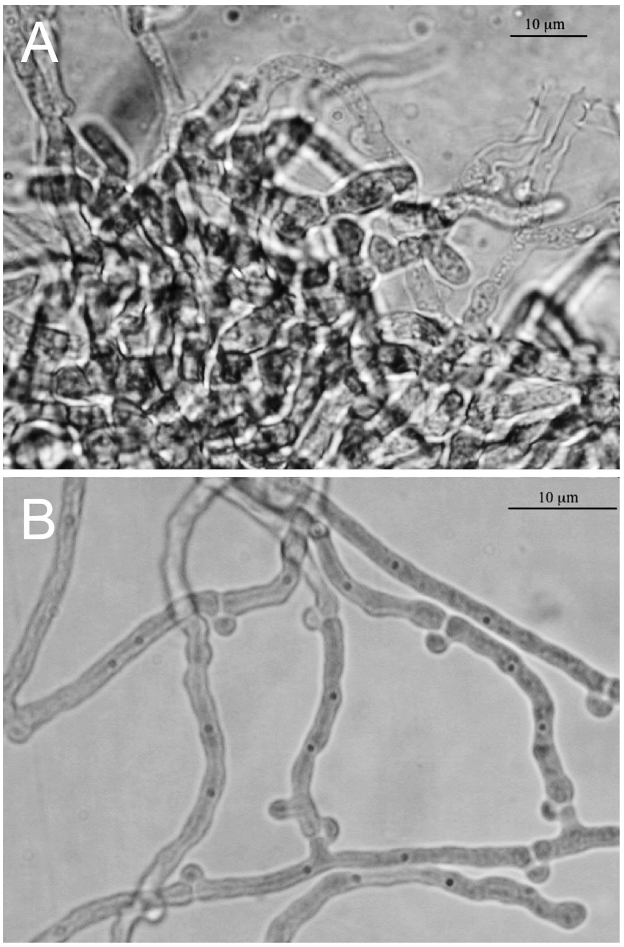

FIG. 3. Sclerotial-like structures (A) and hyphae (B) of isolates obtained from sugar beet at 400× magnification and stained with 0.6% phenosafranin and 3% KOH.

(FIG. 3B) and mean cell width of 2.75 ± 1.2 µm (SD = 0.25).

Production of phenol oxidases.—All isolates produced a dark brown pigment on the underside of the fungal colony when grown on PDA or MEA amended with 0.5% tannic acid (data not shown). No dark brown pigments were observed on the upper or underside of the fungal colony when grown on PDA or MEA without amendment with 0.5% tannic acid. All isolates produced green, orange and red pigments in the presence of gum guiac, guiacol and syringaldezine 15 min after applying the solutions (data not shown).

Hyphal growth temperature experiments.—Data for the two temperature growth experiments were combined for analysis at all temperatures because variances were homogeneous (P = 0.2194–0.9613) and the experimental runs were not significantly different (P = 0.0781–0.9401). The 10 representative isolates averaged 12.0, 27.2, 30.3, 20.2 and 0.0 mm growth at 4, 10, 16, 22 and 28 C respectively (TABLE II). Based on regression analysis, the optimum growth of the isolates from sugar beet was estimated at 14.5 C (FIG. 4). Significant differences ($P < 0.0001$) were evident between isolates at all temperatures, except 28 C where no growth occurred. Isolate F576 was consistently among the slowest growing isolates at 4–22 C, while isolates F566, F567, F568, F574 and F580 consistently exhibited a greater rate of growth. After 2 wk at 10, 16 and 22 C most isolates covered the surface of the 9 cm plate and analysis at these temperatures was not reported. No growth was evident at 28 C after 3 wk, but when these isolates were moved to 22 C growth resumed.

TABLE II. Comparison of representative sample of 10 isolates after 1 wk incubation at 4, 10, 16, 22 and 28 C on potato dextrose agar

	Growth (mm)[a]				
	4 C	10 C	16 C	22 C	28 C
F566	14.6 a	29.6 a	31.5 ab	18.5 c	0.0
F568	13.9 ab	30.2 a	32.8 a	23.0 ab	0.0
F580	13.2 a–c	29.0 ab	33.0 a	18.5 c	0.0
F571	13.1 a–d	28.9 ab	29.6 bc	23.4 ab	0.0
F572	12.1 b–d	27.0 bc	31.1 ab	24.2 a	0.0
F583	11.6 cd	29.0 ab	31.5 ab	22.0 ab	0.0
F575	11.2 c–e	23.1 de	29.9 bc	20.8 bc	0.0
F578	11.1 de	29.0 ab	28.2 c	18.0 c	0.0
F579	9.2 ef	24.9 cd	28.2 c	20.4 bc	0.0
F576	9.0 f	21.0 e	24.2 d	13.4 d	0.0
Overall mean	12.0	27.2	30.3	20.2	0.0
$P > F$[b]	<0.0001	<0.0001	<0.0001	<0.0001	NA
LSD ($P < 0.05$)	2.1	2.3	2.3	3.1	NA

[a] Growth from fungal plug to leading edge of culture.

[b] $P > F$ was the probability associated with the F value. LSD = Fisher's protected least significant difference. Within each temperature, means followed by the same letter did not differ based on Fisher's protected LSD. NA = no analysis since there was no fungal growth. Data for the two experiments were combined for analysis at all temperatures since variances were homogeneous (P = 0.2194–0.9613) and the experiments were not significantly different (P = 0.0781–0.9401).

Amplification and sequencing of rDNA.—A single fragment of ITS and 5′ end of the LSU rDNA was successfully amplified from 15 of the 18 sugar beet isolates with approximately 700 and 1400 bp respectively. PCR products of ITS and partial LSU were not obtained from isolates F567, F569 and F574 because of poor mycelial growth and/or the failure of DNA extraction and PCR. Sequences of the 5′ end of the

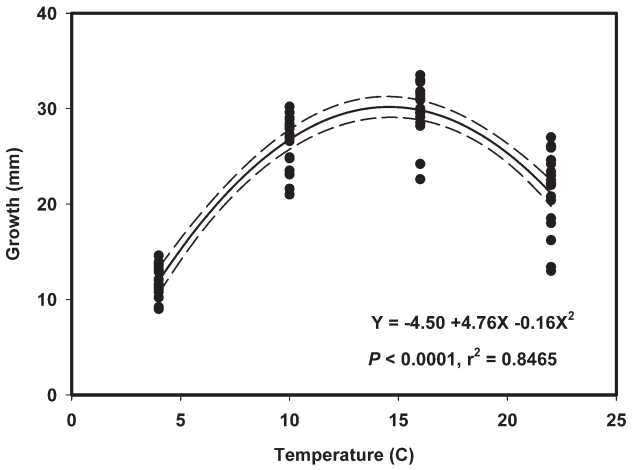

FIG. 4. Solid regression line for growth of 18 basidiomycete isolates from sugar beet roots after incubation 1 wk at 4, 10, 16 and 22 C on potato dextrose agar. Optimum hyphal growth was estimated to occur at 14.5 C. Dashed lines represent the 95% confidence limits.

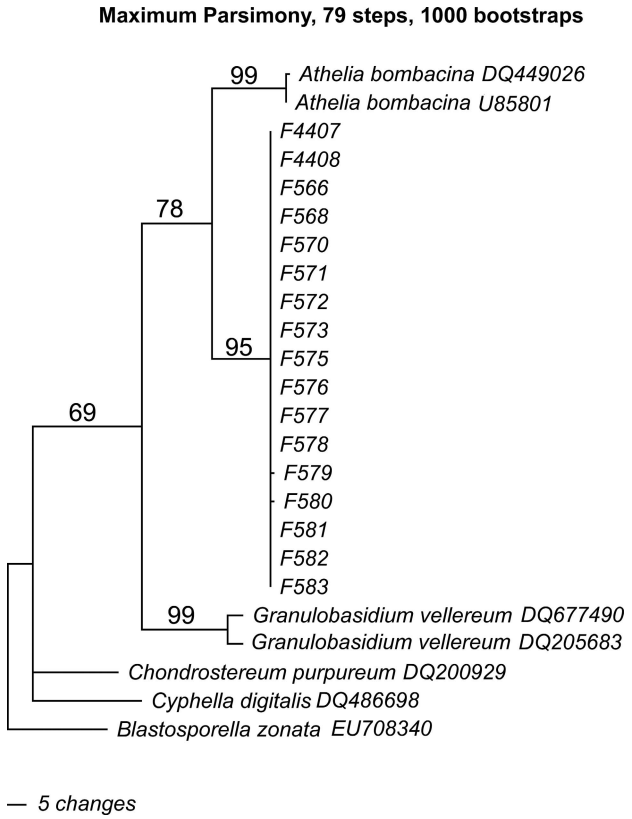

FIG. 5. Maximum parsimony (MP) phylogeny of sugar beet isolates based on sequence analysis of the nuclear large subunit rDNA gene. Statistical support was calculated from 1000 bootstrap replicates. Accession numbers for those sequences publicly available in NCBI are to the right of the species name. The sugar beet isolates are depicted with the letter F followed by the identifying number.

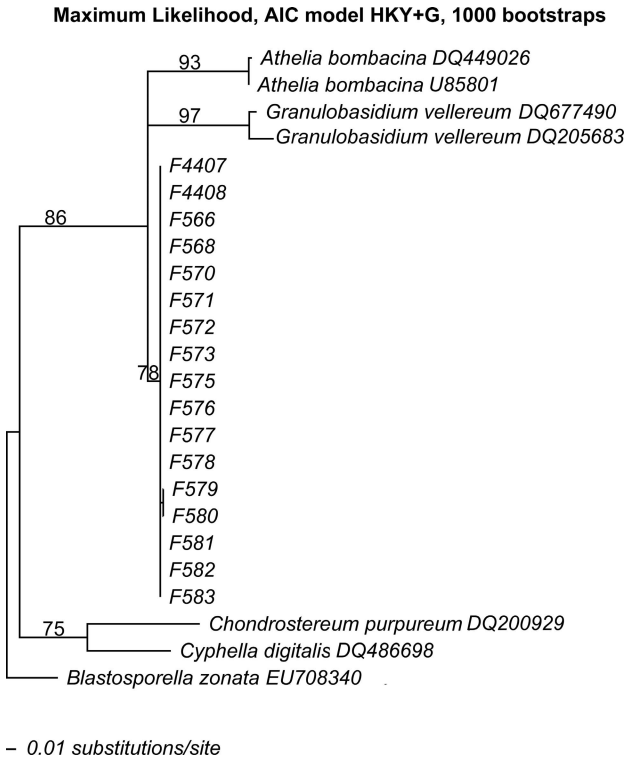

FIG. 6. Maximum likelihood (ML) phylogeny of sugar beet isolates based on sequence analysis of the nuclear large subunit rDNA gene. Statistical support was calculated from 1000 bootstrap replicates. Accession numbers for those sequences publicly available in NCBI are to the right of the species scientific name. The sugar beet isolates are depicted with the letter F followed by the identifying number.

LSU rDNA from all sugar beet isolates were identical. Sequences of the ITS region from each isolate were nearly identical (99.2–100%). Four substitutions were observed among ITS sequence of five isolates. Each substitution occurred in different nucleotide of the ITS region, except for one nucleotide in ITS2 region of isolates F579 and F580. To better determine the identity of the sugar beet isolates the sequences from the sugar beet isolates were compared to sequences in the NCBI database with BLASTn. BLASTn hits showed that ITS sequences of our sugar beet isolates had 98% sequence identity with those of from an uncultured basidiomycete (isolate dfmo0726_048, accession number AY969953) sampled from soil in North Carolina and a cultured basidiomycete obtained from apple leaves (EU098119) in Switzerland. Maximum likelihood and maximum parsimony analyses based on the ITS and LSU sequences suggest that three fungi in the Basidiomycota (*Athelia bombacina*, *Granulobasidium vellereum*, *Cyphella digitalis*) are phylogentically related to the isolates from sugar beet (FIGS. 5–8). High statistical support for both loci

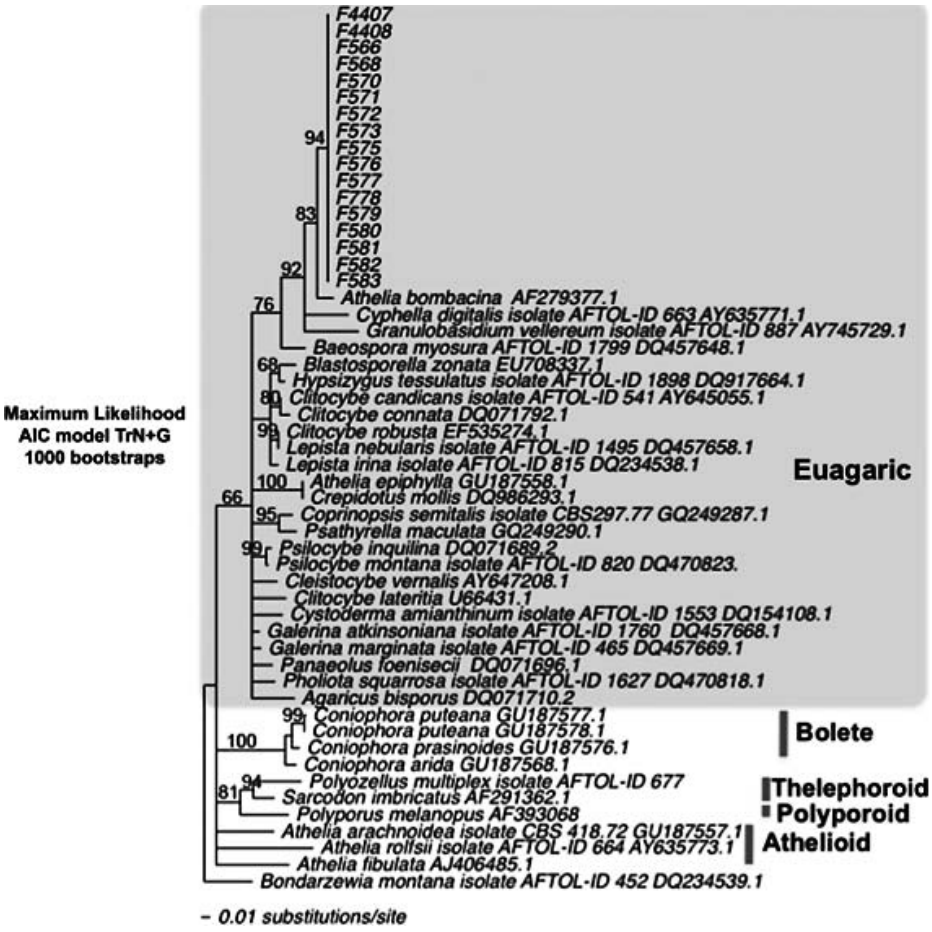

FIG. 7. Maximum parsimony phylogeny of isolates from sugar beet based on the internal transcribed spacer region (ITS) regions of the nuclear encoded rDNA gene. Statistical support was calculated from 1000 bootstrap replicates. Accession numbers for those sequences publicly available in NCBI are to the right of the species scientific name. The sugar beet isolates are depicted with the letter F followed by the serial number.

under different criteria confirmed that *Athelia bombacina* was consistently the closest known *Athelia* species to the sugar beet isolates (FIGS. 7–8).

DISCUSSION

Fungal microbial growth and decay on sugar beet in storage primarily has been attributed to fungi in the Ascomycota, such as *Phoma betae* Frank, *Penicillium claviforme* Bainier and *Botrytis cinerea* Pers. (Bugbee 1982). Fungi of lesser importance associated with storage rot of sugar beet roots also include species of *Aspergillus*, *Alternaria*, *Chaetomium*, *Fusarium*, *Mucor*, *Penicillium*, *Rhizopus*, *Sclerotinia* and *Stemphylium* (Harveson et al. 2009). However, our initial observations of mycelium with clamp connections on hyphal septa sampled from stored sugar beets suggested that the unknown fungus belonged to the Basidiomycota. The occurrence of fungi in the Basidiomycota on stored sugar beet roots is limited to *Armillaria mellea* (Vahl.: Fr.) P. Kumm, *Rhizoctonia solani* Kuhn (teleomorph *Thanatephorus cucumeris* (A.B. Frank) Donk), *Rhizoctonia zeae* Voorhees (teleomorph *Waitea circinata* Warcup & Talbot), *Sclerotium rolfsii* Sacc (teleomorph *Athelia rolfsii*) and *Thanatophytum crocorum* (Pers.: Fr.) Nees (syn. *Rhizoctonia crocorum*; teleomorph *Helicobasidium purpureum* Pat.) (Conners 1967, Grand 1985, Harveson et al. 2009). *Corticium centrifugum* also has been reported on sugar beet (Tai 1979). However, none of these basidiomycete fungi have been associated previously with storage rot on sugar beet in the United States (Bugbee 1982, Harveson et al. 2009).

Isolates obtained from stored sugar beet roots in Idaho did not produce asexual or sexual spores in vivo or in vitro but had binucleate hyphal cells with clamp connections. They formed a dark brown pigment when grown on PDA or MEA amended with 0.5% tannic acid, and color changes were observed when solutions of gum guiac guiacol and syringalde-

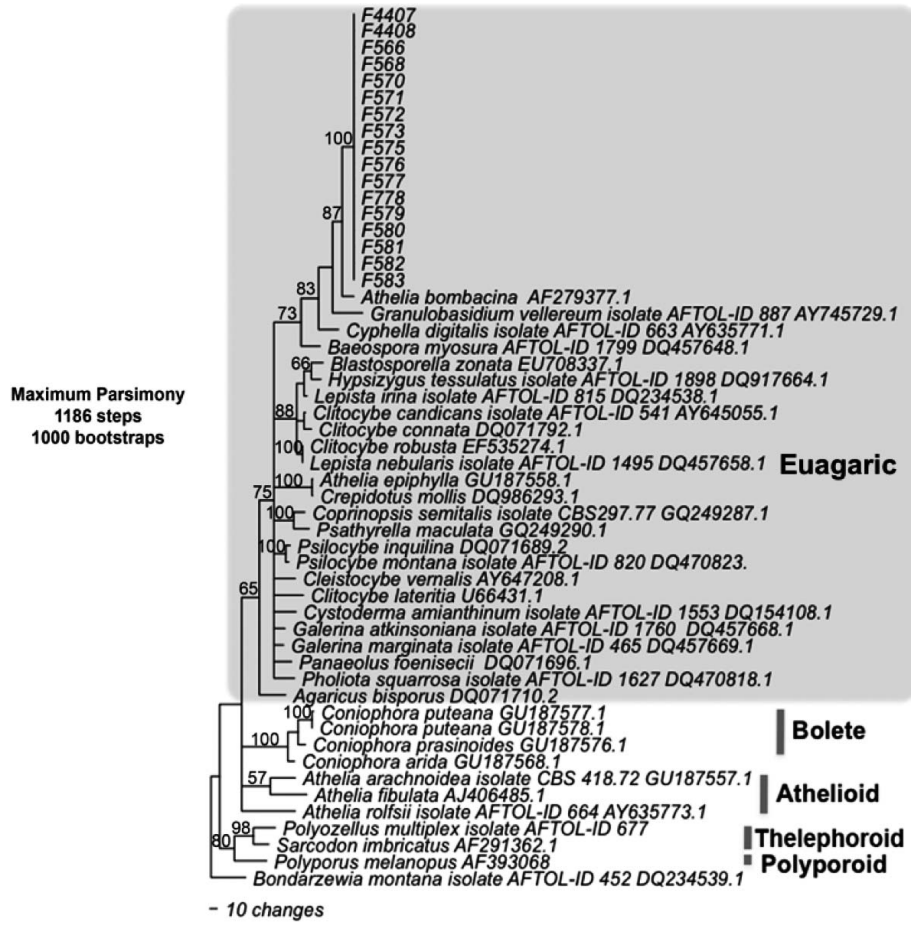

FIG. 8. Maximum likelihood phylogeny of isolates from sugar beet based on the internal transcribed spacer (ITS) region of the nuclear encoded rDNA gene. Statistical support was calculated from 1000 bootstrap replicates. Accession numbers for those sequences publicly available in NCBI are to the right of the species scientific name. The isolates are depicted with the letter F followed by the serial number.

zine were applied directly to mycelium, suggesting that the fungus produced phenol oxidases. The majority of isolates produced orange-brown, sclerotial-like structures every 4–5 d on the surface of PDA and MEA. These structures often formed in a circular pattern in the fungal colony after 4–10 mm radial mycelial growth (FIG. 2A, B).

The unknown fungus from sugar beet roots grew 4–22 C on nutrient medium with an estimated optimal rate of hyphal growth at 14.5 C and no growth at 28 C. Despite morphological differences with the isolates from sugar beet roots, several Basidiomycete species have similar mycelial growth rates to the sugar beet isolates (de Vries et al. 2008). The basidiomycete fungus *Corticium centrifugum* sensu Butler (described as *Butlerelfia eustacei* Weres. & Illman) has been reported to occur on stored apples and pears and exhibits optimum growth at 15–25 C, with slow hyphal growth at 10 C (Weresub and Illman 1980). The cold-tolerant fungus *Athelia arachnoidea* (Berk.) Jülich, (anamorph *Rhizoctonia carotae* Rader) has been identified on carrots and exhibits optimum hyphal growth at 10–21 C (Punja 1987, Ricker and Punja 1991) and 18–23 C (Adams and Kropp 1996). *Fibulorhizoctonia psychrophila* Stalpers & R.P. de Vries also has been identified on carrots with optimum hyphal growth at 9–12 C (de Vries et al. 2008). Both *A. arachnoidea* and *F. psychrophila* were observed on carrot roots after 4–6 mo storage at 1–2 C in high relative humidity (Ricker and Punja 1991, Punja 1987). *F. psychrophila* recently was identified as an anamorph of *Athelia*. Because *Athelia*, *Corticium* and *Fibulorhizoctonia* are associated with low temperature storage similar to indoor sugar beet treatment in Idaho we hypothesized that the fungus associated with stored sugar beet roots in Idaho might be related to these genera based on sequence analysis of the ITS region of the rDNA.

Subsequent analysis of the ITS rDNA sequences indicated that the sugar beet isolates in our study

were different from *A. arachnoidea* (65.3% identity) and *F. psychrophila* (65.5% identity) based on BLASTn and GENETYX-WIN results (data not shown). BLASTn and GENETYX-WIN identified a basidiomycete fungus from apple leaves (EU098119) in Switzerland and an uncultured environmental sample (AY969953) from soil in North Carolina with 98.2% and 98.0% identity respectively to our ITS sequences. These two fungi appear to be more closely related to the sugar beet isolates, while *A. arachnoidea* and *F. psychrophila* are more distantly related. We obtained the culture of the fungus isolated from apple leaves from Switzerland from Julien Crovadore. This isolate had binucleate hyphals cells with clamp connections and produced phenol oxidases but differed morphologically from the sugar beet isolates from Idaho (white-gray colony without the characteristic sclerotial-like structures), hyphal width and temperature growth rate and optimum (data not shown). It is possible that the isolate from apple in Switzerland represents a morphological variant of the sugar beet isolates, but no SSU rDNA sequence was publically available and we were unsuccessful in sequencing this region of the genome for this isolate. Therefore the isolate from apple in Switzerland was not subjected to further sequence analysis. The similarity of the uncultured basdiomycete from North Carolina provides a unique opportunity to isolate the fungus with a semiselective medium from soil at the site where the sequence was derived and to associate this environmental sequence with a living culture.

Maximum likelihood and maximum parsimony analysis conducted on the ITS and LSU sequence data suggest that *Athelia bombacina*, *Granulobasidium vellereum* and *Cyphella digitalis* are phylogenetically related to the isolates from sugar beet (FIGS. 5–8). The taxonomic placement of *G. vellereum* or its close relatives is not known. Although *C. digitalis* was shown to cluster within the *Antrodia* clade, this topology was not strongly supported and the fungus was classified within the polypores (Bodensteiner et al. 2004, Binder et al. 2005). *A. bombacina*, *G vellerum* and *C. digitalis* have been reported to associate with wood, and based on the current phylogeny these fungi appear to be part of the Euagaric clade (Eslyn 1962, Kuntze 1891, Farr et al. 1995). Of interest, *A. bombacina*, *G vellerum* and *C. digitalis* are not part of the core Athleloid clade composed of *Athelia arachnoidea*, *A. fibulata* and *A. rolfsii* as described by Binder et al. (2005) and Larsson (2004). Furthermore, in the current phylogeny *A. epiphylla* also clusters with the Euagarics. The current phylogeny in agreement with studies based on rDNA and EF1a gene shows that the *Athelia* genus is polypheletic (Binder et al. 2005, Larsson 2004, Hibbett et al. 2007, Hibbett and Binder 2002, Matheny et al. 2007). Morphological taxonomy within the *Athelia* species complex has been defined based on the structure of their hyphae with clamp connections, cottony whitish mycelia and globose brownish sclerotia (Adams and Knopp 1996, de Vries et al. 2008). Additional molecular data will be required to determine phylogenetic placement of the sugar beet isolates given that their closest known relative is *Athelia bombacina* and current lack of resolution of the *Athelia* genus. Therefore, increased resolution of the genus *Athelia* is required before proposing a genus and species epithet for isolates from stored sugar beet.

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