Green Mold Diseases of *Agaricus* and *Pleurotus* spp. Are Caused by Related but Phylogenetically Different *Trichoderma* Species

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ABSTRACT

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Producers of champignon (*Agaricus bisporus*) and oyster mushroom (*Pleurotus ostreatus*) are facing recent incidents of green mold epidemics in Hungary. We examined 66 *Trichoderma* strains isolated from *Agaricus* compost and *Pleurotus* substrate samples from three Hungarian mushroom producing companies by a polymerase chain reaction-based diagnostic test for *T. aggressivum*, sequence analysis of the internal transcribed spacer region 1 (ITS1) and ITS2 and (selectively) of the fourth and fifth intron of translation elongation factor 1 α (*tef1 α*), and restriction fragment length polymorphism of mitochondrial DNA. Seven *Trichoderma* species were identified: *T. aggressivum* f. *europaeum* (17

isolates), *T. harzianum* (three isolates), *T. longibrachiatum* (four isolates), *T. ghanense* (one isolate), *T. asperellum* (four isolates), *T. atroviride* (nine isolates), and a still undescribed phylogenetic species, *Trichoderma* sp. DAOM 175924 (28 isolates). *T. aggressivum* f. *europaeum* was exclusively derived from *A. bisporus* compost, whereas *Trichoderma* sp. DAOM 175924 exclusively occurred in the substrate for *Pleurotus* cultivation. Sequences of the latter strains were co-specific with those for *Trichoderma* pathogens of *P. ostreatus* in Korea. The widespread occurrence of this new species raises questions as to why infections by it have just only recently been observed. Our data document that (i) green mold disease by *T. aggressivum* f. *europaeum* has geographically expanded to Central Europe; (ii) the green mold disease of *P. ostreatus* in Hungary is due to the same *Trichoderma* species as in Korea and the worldwide distribution of the new species indicates the possibility of spreading epidemics; and (iii) on mushroom farms, the two species are specialized on their different substrates.

Worldwide mushroom cultivation is dominated by the production of *Agaricus bisporus*, *Lentinula edodes*, and *Pleurotus ostreatus* (5). For the first, the association of *Trichoderma* species with the respective compost has been known for a long time to limit commercial production (25). However, this problem became severe with the onset of green mold epidemic—or epimycotic—in Northern Ireland in 1985, which was quickly succeeded by subsequent outbreaks in Ireland (1986), England and Scotland (1987), the Netherlands (1994), France (1997), and Spain (1998) (10,15,17,18). In the early 1990s, a similar disease appeared in mushroom crops in the United States and Canada (4,19,20). The escalation of green mold evoked extensive research efforts to identify and study the causative agent. Although a number of *Trichoderma* spp. have been isolated from mushroom compost, aggressive colonization was shown to be exclusively due to two forma speciales of a new species of *Trichoderma*, viz. *T. aggressivum* f. *europaeum* in Europe and *T. aggressivum* f. *aggressivum* in North America (23), which have previously been described as *T. harzianum* biotypes 2 and 4, respectively (17,18).

In the last 2 years, outbreaks of *Trichoderma* green mold have also been reported to seriously affect the commercial production of *P. ostreatus* in Korea (21) and Italy (26). The causative agent appears to be distinct from *T. aggressivum* in morphological and

cultural characters (21,26). Park et al. (21) claimed that two different new species (*T. koreana* and *T. pleuroti*) are responsible for the epidemics on *P. ostreatus* in Korea, but unfortunately they did not document the data allowing this conclusion.

Hungary is one of the leading European nations for mushroom production and export (38,000 and 16,000 tons in the year 2000, respectively; source, Hungarian Vegetable and Fruit Board). Recently, Hungarian producers noticed the occurrence of a *Trichoderma* green mold problem in their mushroom farms both for *A. bisporus* and for *P. ostreatus*, suggesting the simultaneous emergence of aggressive *Trichoderma* strains. In the present study, we addressed the following questions arising from this situation. (i) Is the green mold disease of *A. bisporus* due to *T. aggressivum* f. *europaeum*, i.e., has the Western European epidemic now spread to Central Europe? (ii) Is the green mold disease of *P. ostreatus* in Hungary due to *T. aggressivum* or to the *Trichoderma* species reported from Korea? (iii) And are these two types of green mold specific for their substrates?

MATERIALS AND METHODS

Strains and culture conditions. *Trichoderma* strains were isolated from samples of compost and substrate used for *A. bisporus* and *P. ostreatus* cultivation from three Hungarian mushroom farms (referred to further as A, B, and C). Farm A produces *P. ostreatus* on wheat straw as substrate, while farm B produces *A. bisporus* on compost containing wheat straw, chicken manure, horse manure, and gypsum, supplemented with soya meal as an

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additive. Farm C produces both mushrooms on the corresponding substrates described above. Samples were put on plates containing solid yeast extract-glucose (YEG) medium (5 g of glucose per liter, 1 g of yeast extract per liter, 5 g of KH_2PO_4 per liter, and 20 g of agar per liter in distilled water supplemented with 0.1 g of streptomycin per liter and 0.1 g of chloramphenicol per liter). After the occurrence of conidiation, conidial suspensions were prepared, diluted, and plated on solid YEG medium. Agar plugs cut from separately growing young *Trichoderma* colonies were transferred to new plates with the same medium. The isolates were deposited in the strain collection of the University of Szeged, Hungary, and are available from the corresponding author.

Polymerase chain reaction-based identification of *T. aggressivum*. The diagnostic polymerase chain reaction (PCR) test for *T. aggressivum* developed by Chen et al. (6) was carried out with primers Th-F (5'-CGGTGACATCTGAAAAGTCGTG-3') and Th-

R (5'-TGTCACCCGTTTCGGATCATCCG-3') in a T3 thermocycler (Biometra, Gottinger, Germany) with 1 cycle at 94°C for 2 min, 35 cycles at 94°C for 15 s, 62°C for 30 s, and 74°C for 30 s, and a final elongation step at 72°C for 7 min.

Amplification and sequence analysis of the ITS region and *tef1*. DNA isolation, PCR amplification of the internal transcribed spacer (ITS1-5.8S rDNA-ITS2) region, and automatic DNA sequencing of the fragments were carried out as described previously (22). A 0.7-kb fragment of the translation elongation factor 1 α (*tef1*), containing the fourth and fifth intron, was amplified by the primer pair EF1-728F (5'-CATCGAGA-AGTTCGAGAAGG-3') and TEF-LLErev (5'-AACTTGCAGGCA-ATGTGG-3'). Amplification was performed in a T3 thermocycler as follows: 1 cycle at 94°C for 1 min, 30 cycles at 94°C for 1 min, 59°C for 1 min, and 74°C for 50 s, and a final elongation step at 74°C for 7 min. Amplicon purification and sequencing was done

TABLE 1. Strains, sources of isolation, and GenBank accession numbers of internal transcribed spacer 1 (ITS1), ITS2, and *tef1* sequences

Strain	Source of isolation	Taxon	ITS1	ITS2	<i>tef1</i>
CBS 450.95, CBS 100527, CBS 100528	<i>Agaricus</i> compost, North America	<i>T. aggressivum</i> f. <i>aggressivum</i>	DQ328880	DQ328904	
CBS 100525, CBS 100526, CBS 433.95, CBS 689.94	<i>Agaricus</i> compost, United Kingdom	<i>T. aggressivum</i> f. <i>europaeum</i>	DQ328879	DQ328903	
A03	<i>Pleurotus</i> substrate, Farm A, Hungary	<i>Trichoderma</i> sp. DAOM 175924	DQ328882 ^a	DQ328912	
A06	<i>Pleurotus</i> substrate, Farm A, Hungary	<i>Trichoderma</i> sp. DAOM 175924	DQ328882 ^a	DQ328911	
A08	<i>Pleurotus</i> substrate, Farm A, Hungary	<i>Trichoderma</i> sp. DAOM 175924	DQ328897	DQ328939	
A09, A13, A33	<i>Pleurotus</i> substrate, Farm A, Hungary	<i>Trichoderma</i> sp. DAOM 175924	DQ328882	DQ328907	
A11	<i>Pleurotus</i> substrate, Farm A, Hungary	<i>Trichoderma</i> sp. DAOM 175924	DQ328898	DQ328939	
A14	<i>Pleurotus</i> substrate, Farm A, Hungary	<i>Trichoderma</i> sp. DAOM 175924	DQ328882	DQ328913	
A15, A20, A22, A23, A27, A30, A34	<i>Pleurotus</i> substrate, Farm A, Hungary	<i>Trichoderma</i> sp. DAOM 175924	DQ328882	DQ328915	
A16	<i>Pleurotus</i> substrate, Farm A, Hungary	<i>Trichoderma</i> sp. DAOM 175924	DQ328882	DQ328906	
A18	<i>Pleurotus</i> substrate, Farm A, Hungary	<i>Trichoderma</i> sp. DAOM 175924	DQ328882	DQ328914	
A19	<i>Pleurotus</i> substrate, Farm A, Hungary	<i>Trichoderma</i> sp. DAOM 175924	DQ328883	DQ328906	
A25	<i>Pleurotus</i> substrate, Farm A, Hungary	<i>Trichoderma</i> sp. DAOM 175924	DQ328898	DQ328939	
A26	<i>Pleurotus</i> substrate, Farm A, Hungary	<i>Trichoderma</i> sp. DAOM 175924	DQ328888	DQ328907	
A28	<i>Pleurotus</i> substrate, Farm A, Hungary	<i>Trichoderma</i> sp. DAOM 175924	DQ328884	DQ328907	
A29	<i>Pleurotus</i> substrate, Farm A, Hungary	<i>Trichoderma</i> sp. DAOM 175924	DQ328882	DQ328916	
A35	<i>Pleurotus</i> substrate, Farm A, Hungary	<i>T. longibrachiatum</i>	DQ328902	DQ328943	DQ364636
A37	<i>Pleurotus</i> substrate, Farm A, Hungary	<i>Trichoderma</i> sp. DAOM 175924	DQ328898	DQ328940	
A38	<i>Pleurotus</i> substrate, Farm A, Hungary	<i>T. asperellum</i>	DQ328889	DQ328917	
A39	<i>Pleurotus</i> substrate, Farm A, Hungary	<i>T. atroviride</i>	DQ328890	DQ328918	
B01	<i>Agaricus</i> compost, Farm B, Hungary	<i>T. aggressivum</i> f. <i>europaeum</i>	DQ328892	DQ328903	
B02	<i>Agaricus</i> compost, Farm B, Hungary	<i>T. aggressivum</i> f. <i>europaeum</i>	DQ328899	DQ328903	
B03, B04, B07, B12, B18, B31, B35	<i>Agaricus</i> compost, Farm B, Hungary	<i>T. aggressivum</i> f. <i>europaeum</i>	DQ328879	DQ328903	
B05	<i>Agaricus</i> compost, Farm B, Hungary	<i>T. aggressivum</i> f. <i>europaeum</i>	DQ328879	DQ328921	
B08	<i>Agaricus</i> compost, Farm B, Hungary	<i>T. aggressivum</i> f. <i>europaeum</i>	DQ328900	DQ328903	
B09	<i>Agaricus</i> compost, Farm B, Hungary	<i>T. aggressivum</i> f. <i>europaeum</i>	DQ328879	DQ328922	
B10	<i>Agaricus</i> compost, Farm B, Hungary	<i>T. aggressivum</i> f. <i>europaeum</i>	DQ328879	DQ328923	
B11	<i>Agaricus</i> compost, Farm B, Hungary	<i>T. aggressivum</i> f. <i>europaeum</i>	DQ328893	DQ328903	
B13	<i>Agaricus</i> compost, Farm B, Hungary	<i>T. aggressivum</i> f. <i>europaeum</i>	DQ328879	DQ328925	
B14	<i>Agaricus</i> compost, Farm B, Hungary	<i>T. ghanense</i>	DQ328894	DQ328924	
B15	<i>Agaricus</i> compost, Farm B, Hungary	<i>T. atroviride</i>	DQ328885	DQ328908	
B17	<i>Agaricus</i> compost, Farm B, Hungary	<i>T. longibrachiatum</i>	DQ328886	DQ328909	DQ364633
B19	<i>Agaricus</i> compost, Farm B, Hungary	<i>T. longibrachiatum</i>	DQ328902	DQ328942	
B20	<i>Agaricus</i> compost, Farm B, Hungary	<i>T. atroviride</i>	DQ328890	DQ328926	DQ364634
B21	<i>Agaricus</i> compost, Farm B, Hungary	<i>T. atroviride</i>	DQ328890	DQ328927	
B22	<i>Agaricus</i> compost, Farm B, Hungary	<i>T. atroviride</i>	DQ328890	DQ328929	
B23	<i>Agaricus</i> compost, Farm B, Hungary	<i>T. asperellum</i>	DQ328889	DQ328928	
B24	<i>Agaricus</i> compost, Farm B, Hungary	<i>T. atroviride</i>	DQ328895	DQ328930	
B26	<i>Agaricus</i> compost, Farm B, Hungary	<i>T. asperellum</i>	DQ328889	DQ328936	
B27	<i>Agaricus</i> compost, Farm B, Hungary	<i>T. atroviride</i>	DQ328890	DQ328931	
B28	<i>Agaricus</i> compost, Farm B, Hungary	<i>T. atroviride</i>	DQ328890	DQ328932	
B29	<i>Agaricus</i> compost, Farm B, Hungary	<i>T. longibrachiatum</i>	DQ328887	DQ328910	DQ364635
B30	<i>Agaricus</i> compost, Farm B, Hungary	<i>T. atroviride</i>	DQ328890	DQ328933	
B32	<i>Agaricus</i> compost, Farm B, Hungary	<i>T. aggressivum</i> f. <i>europaeum</i>	DQ328879	DQ328934	
B34	<i>Agaricus</i> compost, Farm B, Hungary	<i>T. aggressivum</i> f. <i>europaeum</i>	DQ328879	DQ328935	
B37	<i>Agaricus</i> compost, Farm B, Hungary	<i>T. asperellum</i>	DQ328889	DQ328937	
C01	<i>Agaricus</i> compost, Farm C, Hungary	<i>T. harzianum</i>	DQ328891	DQ328919	
C02	<i>Pleurotus</i> substrate, Farm C, Hungary	<i>Trichoderma</i> sp. DAOM 175924	DQ328882	DQ328920	
C03	<i>Pleurotus</i> substrate, Farm C, Hungary	<i>Trichoderma</i> sp. DAOM 175924	DQ328896	DQ328938	
C08	<i>Agaricus</i> compost, Farm C, Hungary	<i>T. harzianum</i>	DQ328901	DQ328941	
C15	<i>Pleurotus</i> substrate, Farm C, Hungary	<i>Trichoderma</i> sp. DAOM 175924	DQ328898	DQ328939	
C22	<i>Agaricus</i> compost, Farm C, Hungary	<i>T. harzianum</i>	DQ328881	DQ328905	
C25, C27	<i>Pleurotus</i> substrate, Farm C, Hungary	<i>Trichoderma</i> sp. DAOM 175924	DQ328897	DQ328939	

^a Identical GenBank accession numbers indicate that the sequences of the corresponding isolates are identical.

as described previously (13). All sequences obtained in this study have been submitted to NCBI GenBank and their accession numbers are indicated in Table 1.

Identification of *Trichoderma* at the species level. Sequence analysis of ITS1 and ITS2 and *tefl* was performed with the aid of the programs *TrichOKey* 1.0 (8) and *TrichoBLAST* (12) available online by the International Subcommission on *Trichoderma* and *Hypocrea* Taxonomy.

Restriction fragment length polymorphism analysis of mitochondrial DNA. Restriction fragment length polymorphisms (RFLPs) of mitochondrial DNA (mtDNA) were generated with *Hin6I* as described by Antal et al. (2). The mtDNA profiles were converted to a similarity matrix and phylogenetic distances were calculated with the PhylTools software (3) to create a dendrogram by the unweighted pair group method with arithmetic means using the NEIGHBOR program of PHYLIP version 3.57 (9).

RESULTS

Identification of *Trichoderma* strains from *Agaricus* compost and *Pleurotus* substrate. A total of 66 *Trichoderma* isolates were isolated from compost and substrate samples from three different Hungarian mushroom growing companies: 26 from farm A (producing *P. ostreatus*), 32 from farm B (producing *A. bisporus*), and 8 from farm C (producing both of them). In order to obtain a quick balance of how many of them were *T. aggressivum*, a diagnostic PCR amplification test was performed with the specific primers Th-F and Th-R. This resulted in the tentative identification of 16 isolates as *T. aggressivum* (Fig. 1). However, several other isolates produced faint, yet detectable amplicons, rendering the result of this method uncertain. Therefore, fragments spanning the ITS1 and ITS2 sequences of the rRNA cluster were amplified, sequenced, and subjected to identification by the aid of *TrichOKey* and *TrichoBLAST*. This strategy identified 17 isolates as *T. aggressivum*, thereby confirming all of the identifications reported above and additionally including one isolate that gave only a faint band in the diagnostic assay (Fig. 1, B34). The other 49 *Trichoderma* strains were also identified by means of *TrichOKey*: they included *T. harzianum* (allele of the ex-type culture, three isolates), *T. atroviride* (nine isolates), *T. asperellum* (four isolates), and *T. ghanense* (one isolate). Four isolates belonged to the duplet of species *T. longibrachiatum*/*Hypocrea orientalis*, which exhibits identical ITS1 and ITS2 sequences and cannot be differentiated further by *TrichOKey*. Similarity searches were therefore performed with their *tefl* sequences, which revealed that they belong to the clade of *T. longibrachiatum*. The remaining 27 strains were identified as a yet unnamed phylogenetic species of *Trichoderma*, *Trichoderma* sp. DAOM 175924. This preliminary denomination is based on a strain with the same ITS type isolated from decayed *Acer* stump in Ontario, Canada (13).

Distribution of *Trichoderma* species between *Agaricus* and *Pleurotus* substrates. The distribution of the identified species within the samples derived from cultivation of *A. bisporus* and *P. ostreatus* revealed an interesting pattern. *T. aggressivum* was

exclusively isolated from *A. bisporus* compost, whereas *Trichoderma* sp. DAOM 175924 was exclusively found in the substrate for growth of *P. ostreatus* (Table 2). Other species were found only in minor proportions, although occurring at higher frequency on *Agaricus* compost. The compost for production of *Agaricus* at location C—a farm with no significant *A. bisporus* losses due to *Trichoderma* sp.—was only contaminated by *T. harzianum*. These findings show that on mushroom farms there is a strict specificity of *T. aggressivum* and *Trichoderma* sp. DAOM 175924 for *A. bisporus* compost and *P. ostreatus* substrate, respectively, which is still maintained when the two basidiomycetes are cultivated in close geographic vicinity.

***Trichoderma* sp. DAOM 175924 is co-specific with the *Pleurotus*-pathogenic *Trichoderma* isolates from Korea.** Having demonstrated that the occurrence of green mold in *P. ostreatus* substrate is due to a different *Trichoderma* species than that in *Agaricus* compost (*T. aggressivum*), we wondered whether *Trichoderma* sp. DAOM 175924 would be the same as one of the two species which have recently been reported to be the agent of *Pleurotus* disease in Korea (21). To this end, we retrieved the available ITS1 and ITS2 sequences for *T. koreana* and *T. pleuroti* (accession nos. DQ164405 to DQ164410) and submitted them to identification by *TrichOKey* and *TrichoBLAST*. All of these strains were identified as *Trichoderma* sp. DAOM 175924, and their sequence variation (1 nucleotide in ITS2) fell into the variation seen with other isolates of *Trichoderma* sp. DAOM 175924. We therefore conclude that the *Pleurotus* green mold isolates from Korea represent the same species as those found in the present study in Hungary.

Hungarian *T. aggressivum* isolates display the same mtDNA RFLP pattern as the early UK isolates. In order to learn whether the green mold outbreak observed in Hungary indeed results from spreading of the Western European *Agaricus* green mold epidemic, we compared the mtDNA RFLPs of the strains from this study with those of the *T. aggressivum* f. *europaeum* isolates collected during the epidemic onset in Ireland and England in the late 1980s (24). This comparison showed that both populations share exactly the same RFLP pattern (Fig.

TABLE 2. Distribution of *Trichoderma* species in Hungarian *Agaricus* compost and *Pleurotus* substrate samples

Species	Sample				Total
	A (<i>Pleurotus</i> substrate)	B (<i>Agaricus</i> compost)	C/1 (<i>Pleurotus</i> substrate)	C/2 (<i>Agaricus</i> compost)	
<i>T. harzianum</i>	0	0	0	3	3
<i>Trichoderma</i> sp. DAOM 75924	23	0	5	0	28
<i>T. aggressivum</i>	0	17	0	0	17
<i>T. atroviride</i>	1	8	0	0	9
<i>T. asperellum</i>	1	3	0	0	4
<i>T. longibrachiatum</i>	1	3	0	0	4
<i>T. ghanense</i>	0	1	0	0	1
Total	26	32	5	3	66

Fig. 1. Screening of Hungarian *Trichoderma* isolates from mushroom compost and substrate for *T. aggressivum* by diagnostic polymerase chain reaction. +, positive control (*T. aggressivum* f. *europaeum* CBS 100526); -, negative control without DNA.

2A and C), which was clearly different from that of *T. aggressivum* f. *aggressivum*. For the latter, a common pattern could be observed apart from the small variance resulting from the presence or absence of a 2.2-kb band (Fig. 2A), the derivative of a DNase sensitive, S1 nuclease and RNase resistant plasmid of approximately 5.0 kb, which was detected in the undigested DNA (data not shown).

***Trichoderma* sp. DAOM 175924 displays mtDNA RFLP heterogeneity.** In contrast to *T. aggressivum* f. *europaeum*, isolates of *Trichoderma* sp. DAOM 175924 could be divided into three main distinct groups based on RFLPs of mtDNA (Fig. 2B and C). Samples from location A contained two of these groups, and C, the third. In the course of this investigation, we also

observed the presence of a plasmid-derived 2.2-kb band in some but not all isolates of *Trichoderma* sp. DAOM 175924 (Fig. 2B).

DISCUSSION

In this study, we performed a survey of *Trichoderma* spp. present in the substrates for commercial production of *A. bisporus* and *P. ostreatus* in Hungary. The data obtained for the former are in agreement with those of studies in Western Europe and the United States, i.e., that *T. aggressivum* is the major contaminant of *Agaricus* compost, but that some other species such as *T. atroviride*, *T. asperellum*, *T. harzianum*, and *T. longibrachiatum* can also be found in significant proportions (4). In contrast, and in

Fig. 2. Mitochondrial DNA polymorphism of *Trichoderma aggressivum* and *Trichoderma* sp. DAOM 175924 strains revealed by restriction fragment length polymorphism analysis with the restriction enzyme *Hin*6I. **A**, Lane 1, *T. aggressivum* f. *europaeum* B01; lane 2, *T. aggressivum* f. *europaeum* B07; lane 3, *T. aggressivum* f. *europaeum* B08; lane 4, *T. aggressivum* f. *europaeum* CBS 433.95; lane 5, *T. aggressivum* f. *europaeum* CBS 689.94; lane 6, *T. aggressivum* f. *aggressivum* CBS 450.95; lane 7, *T. aggressivum* f. *aggressivum* CBS 100528; lane 8, *T. aggressivum* f. *aggressivum* CBS 100527; and M, Lambda DNA/*Hind*III marker. **B**, Lane 1: *Trichoderma* sp. DAOM 175924 C15; lane 2, *Trichoderma* sp. DAOM 175924 A08; lane 3, *Trichoderma* sp. DAOM 175924 A16; lane 4, *Trichoderma* sp. DAOM 175924 A28; lane 5, *Trichoderma* sp. DAOM 175924 A37; and M, Lambda DNA/*Hind*III marker. **C**, Dendrogram of *T. aggressivum* and *Trichoderma* sp. DAOM 175924 strains based on their mtDNA profiles. The scale bar represents genetic distance.

agreement with Park et al. (21), the substrate for oyster mushroom cultivation contained almost exclusively a new species of *Trichoderma*, the unnamed phylogenetic species *Trichoderma* sp. DAOM 175924. This indicates differences in the biology of these two *Trichoderma* pathogens, the latter apparently being a strong competitor of other *Trichoderma* spp. In fact, the *Pleurotus* green mold is known to be due to a heavy colonization and sporulation of *Trichoderma* spp. on the substrate, which ultimately inhibits growth of *P. ostreatus*.

There are further significant differences between *T. aggressivum* and *Trichoderma* sp. DAOM 175924: the former was so far isolated only from compost for production of *A. bisporus*, it was never observed in any field study. Seaby (24) speculated that red pepper mites may act as the vector for *T. aggressivum*. Such a mechanism of distribution would be consistent with the slow but steady migration of this infection within Europe and also within the United States, and with the fact that f. *aggressivum* and f. *europaeum* still maintain their allopatric distribution, and renders the compost and the origin of its constituents a major source for the infection. In contrast, isolates of *Trichoderma* sp. DAOM 175924 have widely been found in soil or on decaying wood in the United States, Canada, New Zealand, Europe, India (I. S. Druzhinina and C. P. Kubicek, unpublished data), Iran (D. Zafari, personal communication), and China (27). The widespread occurrence of this new species raises the questions as to why infections by it have just only recently been observed and what makes this species specific for antagonizing just *P. ostreatus*. Interestingly, one of the *Trichoderma* sp. DAOM 175924 isolates (originally identified as "*T. harzianum*" HEND) is used in New Zealand as a biocontrol agent against *Armillaria novae-zealandiae* and *Armillaria limonea* on kiwifruit and pine, respectively (7).

Trichoderma sp. DAOM 175924 was originally misidentified as *T. aureoviride* (14), but molecular analyses proved it to be a new phylogenetic species that forms a sister group to *T. harzianum* and *T. aggressivum* (13). Park et al. (21) recently reported that the Korean *Pleurotus* green mold *Trichoderma* strains represent two distinct new species (*T. koreana* and *T. pleuroti*), which can be distinguished by morphological characters, and sequences of ITS2, *tef1*, and a fragment of the *rpb2* (RNA polymerase II subunit-encoding) gene. Unfortunately, their data and conclusions cannot be evaluated, as most of their sequences and all alignments and phylogenetic trees are unavailable. Only the ITS1-5.8S rDNA-ITS2 sequence of a few isolates could be found in GenBank (accession nos. DQ164405 to DQ164410), and their analysis by *TrichOKey* and *TrichoBLAST* showed that they were identical to isolates of *Trichoderma* sp. DAOM 175924. Park et al. (21) differentiated *T. koreana* and *T. pleuroti* on the basis of an A/C transversion at position 447 in ITS2. The isolates from Hungary contain exclusively C at this position. Sequence analysis of another locus (*tef1*) from several isolates of the C or A type strains also showed phylogenetically relevant nucleotide differences between these two groups of isolates (I. S. Druzhinina and C. P. Kubicek, unpublished data). However, because the names of the two putative species have not been published according to the rules for botanical nomenclature, and most of their gene sequences were not available during this study, we still maintain the designation *Trichoderma* sp. DAOM 175924 until the taxonomy of this species and its eventual split into two species has been clarified.

The analysis of mtDNA, due to its relatively small size and higher mutation rate in comparison with chromosomal DNA, is a powerful tool for differentiation of fungal isolates below the species level. mtDNA RFLP was used earlier to distinguish *Trichoderma* strains isolated from mushroom compost (17,18). Here we applied this method to assess whether the Hungarian isolates of *T. aggressivum* f. *europaeum* belong to the same population as the first isolates from the British Islands, which started the epidemic. The results documented that there are no essential

differences between the Hungarian isolates and the strains from Northern Ireland (CBS 433.95) and England (CBS 100525 and CBS 100526), while they all proved to be clearly different from *T. aggressivum* f. *aggressivum* isolates. This indicates that *T. aggressivum* f. *europaeum* has an essential clonal structure, and that the Hungarian strains likely have been derived from the Western European epidemic lineage. This report therefore indicates that the green mold epidemic of *A. bisporus* also has spread eastward to reach Central Europe.

In contrast to *T. aggressivum*, mtDNA RFLP of the isolates of *Trichoderma* sp. DAOM 175924 revealed at least four different RFLP patterns, three of which were even present in strains isolated from the same *Pleurotus* substrate (sample A), indicating that this species has a much higher genetic variability than *T. aggressivum*. It should be noted that this variability was observed within the ITS2 "C" type, and thus is not proof for the presence of two species within *Trichoderma* sp. DAOM 175924 as suggested by Park et al. (21). It will be interesting, however, to study whether there is a correlation between mtDNA RFLPs and the vigor of infection of *Pleurotus* substrates.

Interestingly, we also detected a plasmid in some but not all Hungarian isolates of *Trichoderma* sp. DAOM 175924. The occurrence of mitochondrial plasmids in *Trichoderma* spp. was reported for *T. viride* (16), *T. aggressivum* f. *aggressivum* (4), and *T. harzianum* (14). In *Fusarium oxysporum* f. *conglutinans*, mitochondrial plasmids have been identified as one of the factors determining the host specificity (11). Further studies are necessary to determine whether the plasmids of *Trichoderma* sp. DAOM 175924 have any effect on its virulence to *P. ostreatus*.

In order to successfully combat the green mold problem, research efforts will be made to examine the physiology of isolates belonging to *Trichoderma* sp. DAOM 175924 and *T. aggressivum* f. *europaeum*, and to reveal the origin of contamination at mushroom farms, which could help the design of efficient control strategies and preventive measures.

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