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CLINICAL IMPORTANCE OF THE GENUS *TRICHODERMA* - A REVIEW*

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Abstract

Opportunistic fungal infections have been occurring with increasing frequency in recent years in immunocompromised patients. Several data were published in the last decade about the clinical importance of the filamentous fungal genus *Trichoderma*, indicating, that *Trichoderma* strains - besides their agricultural and biotechnological importance - may be potential opportunistic pathogens of immunocompromised hosts as well. This review is going to summarize the clinical case reports about *Trichoderma* infections, and discuss the information available on the antifungal susceptibility and on the ecophysiological, enzymological and systematic aspects of clinical *Trichoderma* isolates.

Introduction

Trichoderma species are imperfect filamentous fungi with teleomorphs belonging to the Hypocreales order of the Ascomycota division. The ecological role of this genus is that *Trichoderma* strains take part in the decomposition of plant residues in the soil. Some *Trichoderma* species are very good cellulase producers and therefore they are important for the biotechnological industry [1, 2]. The agricultural importance of the genus is that some *Trichoderma* species possess good antagonistic abilities against plant pathogenic fungi [3-5]. *Trichoderma* strains with effective antagonistic abilities are potential candidates for the biological control of plant diseases [6, 7].

Trichoderma species have been reported as pathogens of reptiles [8, 9], and in increasing number of cases as etiologic agents in human infections. The spectrum of opportunistic fungi is widening as a result of the rising number of immunocompromised patients. The genus *Trichoderma* is already on the growing list of potential fungal pathogens in immunocompromised hosts.

Case reports about *Trichoderma* infections in the literature

Six species of the genus *Trichoderma* (*T. citrinoviride*, *T. harzianum*, *T. koningii*, *T. longibrachiatum*, *T. pseudokoningii* and *T. viride*) have been identified as etiologic agents of infections in immunocompromised patients. Detailed case reports about *Trichoderma* infections are summarized in Table I.

Numerous cases of peritonitis have been reported in peritoneal dialysis patients with *T. harzianum* [10], *T. koningii* [11, 12], *T. longibrachiatum* [13], *T. pseudokoningii* [14], *T. viride* [15, 16] and an unidentified *Trichoderma* species [17]. The causative agent was isolated from the peritoneal effluent in all of these cases. Three of the patients survived [11, 14, 17], the early catheter removal proved to be a successful therapy.

The number of cases of *Trichoderma* infection in immunocompromised transplant recipients is also significant. A fatal infection caused by a *T. pseudokoningii* strain was described in a leukemia patient after bone marrow transplantation. The fungus was isolated from specimens obtained by bronchoalveolar lavage and skin biopsy. Autopsy has shown that the infection was disseminated: hyphae were present in the lungs, brain, heart, stomach and pretracheal abscesses [18]. A fatal disseminated *T. longibrachiatum* infection occurred in a patient who had received an allogenic bone marrow transplant for acute lymphoblastic leukemia. The fungus was recovered from a perirectal ulcer biopsy and stool surveillance cultures, suggesting the gastrointestinal tract

as the portal of entry [19]. A liver transplant recipient developed infection of a perihepatic haematoma due to *T. viride*. The fungus was found to be persistent in the immunocompromised host despite amphotericin B treatment and surgical removal of the infected haematoma, however, the patient died of unrelated complications [20]. The acute invasive sinusitis caused by *T. longibrachiatum* in a liver and small bowel transplant recipient was successfully treated with surgical debridements and amphotericin B followed by oral itraconazole [21]. Guarro et al. detected a systemic *T. harzianum* infection in the autopsy study of a renal transplant recipient [22], the fungus was recovered from abscesses in brain and lung tissues.

The isolation of *T. longibrachiatum* was reported in further cases: from an infection of the skin and subcutaneous tissue in a pediatric patient with severe aplastic anemia and neutropenia [23], from a brain abscess in a leukemic patient with prolonged neutropenia [24], from otitis externa in a child [25] and from a HIV-positive patient (American Type Culture Collection, ATCC 208859, [26]). Another case of *Trichoderma* infection connected with AIDS was reported recently [27]. *T. viride* was found in several specimens of sputum of a patient with pulmonary mycetoma [28] and *Trichoderma* species were involved in cases of mycotic keratitis [29] and endocarditis [30] as well.

There is a lack of published case reports in the case of other clinical *Trichoderma* isolates deposited at the University of Alberta Microfungus Collection and Herbarium (*T. longibrachiatum* UAMH 9515 from the peritoneal effluent of a female and *T. citrinoviride* UAMH 9573 from a peritoneal catheter tip), at the Canadian Collection of Fungal Cultures (*T. pseudokoningii* CCFC 007753 and CCFC 007754 isolated from a bone marrow transplant recipient and from a liver and bowel transplant recipient, respectively), and at Centraalbureau voor Schimmelcultures (*T. longibrachiatum* CBS 446.95 isolated from the lung of a patient, who died). Further clinical *Trichoderma* strains were sent to the the National Reference Center of Mycoses and Antifungal Drugs at the Institute Pasteur: *T. longibrachiatum* IP-92 0647 from a

patient with acute leukemia, IP-94 0958 from a patient who had a lung transplantation, IP-96 0086 from a haematic wound of an otherwise healthy patient, IP-93 1282 isolated from a bronchoalveolar washing in a 2-month bone marrow recipient, IP-93 1792 obtained from a cerebrospinal derivative catheter, IP-94 1510 from infected maxillar sinus, IP-97 0711 from the liquid of a chylothorax and *T. citrinoviride* IP-95 1151 from blood cultures of a patient in aplasia associated with lymphoma). Five *T. longibrachiatum* isolates originated from different patients were received at the Medical Mycology Research Laboratory, Medical College of Virginia, Virginia Commonwealth University between 1996 and 2001, and examined by Espinel-Ingroff along with other emerging pathogenic filamentous fungi [31, 32]. It is also conceivable, that further *Trichoderma* infections have not been documented.

Most of the clinical *Trichoderma* strains involved in infections were isolated from immunocompromised patients. An exception is the case of an inadvertent infusion of *T. viride* to an immunocompetent host with contaminated intravenous fluid. The patient remained well after 24 hours of amphotericin B treatment [33].

Data indicate that members of the genus *Trichoderma* may also be present as the part of the human microflora. Strains were isolated from sputum (*T. koningii* UAMH 473 and UAMH 475) and from sinus ethmoidalis (*T. pseudokoningii* CBS 500.94). Buzina et al. isolated a *T. viride* strain from the nasal mucus of a healthy patient and a *T. inhamatum* strain from a chronic rhinosinusitis (CRS) patient, however, great number of other fungi were found to be associated with CRS and it is still not known, which of them are responsible for the eosinophilic reaction [34]. The possible involvement of *Trichoderma* species in eosinophil activation was already demonstrated: precipitating antibodies against *T. viride* were detected in the sera of patients with acute eosinophilic pneumonia [35, 36]. It is also known that *T. viride* potentiates IgE-related histamine release from human bronchoalveolar cells, suggesting the possible involvement of the genus in allergic reactions [37].

Susceptibility of clinical *Trichoderma* isolates to antifungal compounds

Antifungal susceptibility testing of fungi involved in opportunistic infections is relevant for the choice of adequate therapy. Data available about the susceptibilities of clinical *Trichoderma* isolates - including our Etest results about the susceptibilities of strains *T. citrinoviride* UAMH 9573, *T. koningii* CM 382 ([38], kindly provided by the Centro Nacional de Microbiología, Immunología y Virología Sanitaria, mycology section, Majadahonda, Madrid), *T. longibrachiatum* ATCC 201044 [23], ATCC 208859, UAMH 7955 [21], UAMH 7956 [19] and UAMH 9515 - are summarized in Table II. Different methods were applied for the determination of the minimal inhibitory concentration (MIC) values of the antifungal agents in the available studies [39-44].

Most isolates proved to be resistant to fluconazole and 5-fluorocytosine (5FC), and susceptible or intermediate to amphotericin B, itraconazole, ketoconazole and miconazole, however, amphotericin B resistance [11, 12, 13, 25] and high MIC-levels of itraconazole [22, 25, 38] and ketoconazole [22] were also reported in certain cases. Antimycotic activity with the disc method revealed apparent susceptibility to voriconazole in the case of five examined isolates [38]. The low susceptibility level of some clinical *Trichoderma* isolates to antifungal drugs may cause difficulties in the therapy of infected patients. In cases of suspected or confirmed *Trichoderma* infection, amphotericin B alone or in combination with itraconazole or ketoconazole is suggested as an adequate choice of treatment, the duration of which should be individualized to each case according to the type and extent of the infection and underlying predisposing conditions [23].

Ecophysiological and enzymological investigation of clinical *Trichoderma* isolates

Several data are available about the ecophysiological and enzymological properties of mycoparasitic *Trichoderma* strains with biocontrol potential [45-49], however, there is a lack of similar studies on clinical *Trichoderma* isolates.

We examined the ecophysiological features of strains *T. citrinoviride* UAMH 9573, *T. koningii* CM 382, *T. longibrachiatum* ATCC 201044, ATCC 208859, UAMH 7955, UAMH 7956 and UAMH 9515. The influence of temperature and pH on linear mycelial growth was studied on both minimal and yeast extract agar media [38]. All of the strains were able to grow on these media at temperatures ranging from 10 to 40 °C, with an optimum at 30 °C. Studies on the ecophysiology of the genus *Trichoderma* concluded, that species belonging to the Longibrachiatum section of the genus *Trichoderma* (including *T. citrinoviride* and *T. longibrachiatum*) have higher optimum growth temperatures [26, 50, 51]. Because growth at elevated temperatures is one of the virulence factors of fungi, it is not surprising, that most of the strains involved in *Trichoderma* infections are belonging to the Longibrachiatum section of the genus. The pH ranging from 2.0 to 9.0 supported the growth of most of the examined strains at 25 °C, with an optimum at pH 4. At elevated temperature (37 °C) the range was narrower, but all of the clinical strains were able to grow at physiological pH, which ability can promote their growth as facultative human pathogens [38].

The utilization of 80 compounds as carbon sources, and 34 compounds as nitrogen sources were also investigated. A series of amino acids, e.g. L-asparagine, L-aspartic acid, L-glutamine, L-glutamic acid, L-ornithine, γ -amino-butyric acid and *N*-acetyl-D-glucosamine were utilized by all of the examined clinical *Trichoderma* strains both as carbon and nitrogen source [38].

We examined the extracellular proteolytic enzyme activities in the supernatants of induced cultures of strains *T. citrinoviride* UAMH 9573, *T. koningii* CM 382, *T. longibrachiatum* UAMH 7955, UAMH 7956 and UAMH 9515 by Sephadex G-100 column chromatography [52]. Trypsin-like protease, chymotrypsin-like protease and Leu-aminopeptidase activities cleaving *N*-Benzoyl-Phe-Val-Arg-*p*-nitroanilide, *N*-Succinyl-Ala-Ala-Pro-Phe-*p*-nitroanilide and L-Leu-*p*-nitroanilide, respectively, were found to be common among the examined strains. However, strain *T. koningii* CM 382 has not produced any trypsin-like and chymotrypsin-like proteases and only limited chymotrypsin-like and Leu-aminopeptidase activities were present in the culture supernatant of strain *T. longibrachiatum* UAMH 9515. Sephadex G-100 profiles of these three types of proteolytic enzyme activities proved to be complex suggesting the presence of more isoenzymes. The isoenzyme profiles were found to be dependent on the culturing conditions in many cases. Different Leu-aminopeptidase isoenzymes were secreted by *Trichoderma citrinoviride* UAMH 7956 at pH 5.0 and pH 7.0. In the case of some strains, the activities of certain proteases proved to be higher in the ferment broths of induced cultures shaken at 37 °C than of those shaken at 25 °C. Proteases with such characteristics may be involved in the virulence of opportunistic *Trichoderma* strains [52].

Systematic studies on clinical *Trichoderma* isolates

The appropriate identification of the fungal etiologic agents involved in opportunistic infections is crucial for the adequate choice of specific therapeutic interventions. Several clinical *Trichoderma* isolates were identified on the basis of morphological characteristics [e.g. 12, 18, 22]. As the identification of *Trichoderma* strains is difficult if only morphological characters are considered, the identity of some clinical *Trichoderma* isolates was confirmed by molecular techniques, like DNA-fingerprinting [19, 21, 23], or the sequencing of ribosomal DNA internal

transcribed spacer region ITS-1 – 5.8S - ITS-2 [19, 24, 25]. Kuhls et al. [53] used PCR-fingerprinting to identify six human pathogenic *Trichoderma* isolates - including two strains from reported cases [18, 24] - as *T. longibrachiatum* (5 isolates) and *T. citrinoviride* (1 isolate). Sequencing experiments of the ITS-region confirmed the identifications made on the basis of PCR-fingerprinting data. The results of this study revealed that it is necessary to reidentify a morphologically identified clinical *T. pseudokoningii* strain [18] as *T. longibrachiatum*.

As the most of the clinical *Trichoderma* isolates belong to the section Longibrachiatum of the genus *Trichoderma*, we plan to examine clinical and saprophytic *T. longibrachiatum* and *T. citrinoviride* isolates with the method of Random Amplified Polymorphic DNA (RAPD). Such studies may reveal characteristic RAPD-bands for the discrimination between the section Longibrachiatum and other sections, and between clinical and saprophytic isolates within the Longibrachiatum section, enabling the design of specific primers of diagnostic value for the rapid identification of clinical *Trichoderma* isolates.

Conclusions

In immunocompromised patients, *Trichoderma* strains are able to cause diseases varying from localized infections to fatal disseminated diseases. Most of the cases are reported from patients undergoing peritoneal dialysis and from transplant recipients. *T. longibrachiatum* is the most frequently occurring etiologic agent within the genus. The detailed case reports and data about the antifungal susceptibilities of clinical *Trichoderma* isolates reveal useful information for the planning of the therapy in cases of suspected or confirmed *Trichoderma* infections.

The ecophysiological and enzymological investigation of clinical *Trichoderma* isolates may result in better understanding of their virulence factors. Growth at elevated temperatures, the ability to tolerate neutral pH and to utilize amino acids as carbon and nitrogen sources, as

well as the production of extracellular proteolytic enzymes are among the potential virulence factors of *Trichoderma* strains involved in opportunistic infections. Other factors, like the hydrophobicity of conidia, melanic or carotenoid pigments and mycotoxins are also among the possible virulence factors of fungal opportunists [54].

The identification of *Trichoderma* species is difficult based on morphological characters only, therefore the identity of clinical isolates is suggested to be confirmed by molecular techniques. Furthermore, molecular methods may reveal diagnostic tools for rapid species identification in the clinical practice.

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REFERENCES

1. Réczey,K., Szengyel,Z., Eklund,R., Zacchi,G.: Cellulase production by *T. reesei*. *Bioresource Technol* **57**, 25–30 (1996).
2. Juhász,T., Kozma,K., Szengyel,Z., Réczey,K.: Production of β -glucosidase by mixed culture of *Aspergillus niger* BKMF 1305 and *Trichoderma reesei* RUT C30. *Food Technol Biotechnol* in press (2003).
3. Sivan,A., Chet, I.: Biological control of *Fusarium* spp. in cotton, wheat and muskmelon by *Trichoderma harzianum*. *J Phytopathol* **116**, 39-47 (1986).
4. Naár,Z., Kecskés,M.: A method for selecting *Trichoderma* strains antagonistic against *Sclerotinia minor*. *Microbiol Res* **150**, 239-246 (1995).

5. Naseby,D.C., Pascual,J.A., Lynch,J.M.: Effect of biocontrol strains of *Trichoderma* on plant growth, *Pythium ultimum* populations, soil microbial communities and soil enzyme activities. J Appl Microbiol **88**, 161-169 (2000).
6. Papavizas,G.C.: *Trichoderma* and *Gliocladium*: biology, ecology, and potential for biocontrol. Annu Rev Phytopathol **23**, 23-54 (1985).
7. Manczinger,L., Antal,Z., Kredics,L.: Ecophysiology and breeding of mycoparasitic *Trichoderma* strains. Acta Microbiol Immunol Hung **49**, 1–14 (2002).
8. Foreyt,W.J., Leathers,C.W.: *Trichoderma* sp. infection in the alligator (*Alligator mississippiensis*). J Herpetol **19**, 530-531 (1985).
9. Jacobson,E.R.: Necrotizing mycotic dermatitis in snakes: clinical and pathologic features. J Am Vet Med Assoc **177**, 838-841 (1980).
10. Guiserix,J., Ramdane,M., Finielz,P., Michault,A., Rajaonarivelo,P.: *Trichoderma harzianum* peritonitis in peritoneal dialysis. Nephron **74**, 473-474 (1996).
11. Ragnaud,J.M., Marceau,C., Roche-Bezian,M.C., Wone,C.: Infection peritoneale a *Trichoderma koningii* sur dialyse peritoneale continue ambulatoire. Med Maladies Infect **7**, 402-405 (1984).
12. Campos-Herrero,M.I., Bordes,A., Perera,A., Ruiz,M.C., Fernandez,A.: *Trichoderma koningii* peritonitis in a patient undergoing peritoneal dialysis. Clin Microbiol Newslett **18**, 150-151 (1996).
13. Tanis,B.C., van der Pijl,H., van Ogtrop,M.L., Kibbelaar,R.E., Chang,P.C.: Fatal fungal peritonitis by *Trichoderma longibrachiatum* complicating peritoneal dialysis. Nephrol Dial Transplant **10**, 114-116 (1995).
14. Rota,S., Marchesi,C., Farina,C., de Bièvre,C.: *Trichoderma pseudokoningii* peritonitis in automated peritoneal dialysis patient succesfully treated by early catheter removal. Perit Dial Int **20**, 91-94 (2000).

15. Loeppky,C.B., Sprouse,R.F., Carlson,J.V., Everett,E.D.: *Trichoderma viride* peritonitis. South Med J **76**, 798-799 (1983).
16. Warnock,D.W., Johnson,E.M.: Clinical manifestation and management of hyalohyphomycosis, phaeohyphomycosis and other uncommon forms of fungal infection in the compromised patient. In: Warnock,D.W., Richardson,M.D. (eds): Fungal infection in the compromised patient. Chichester: John Wiley & Sons Ltd, 1991, pp 248-310
17. Bren,A.: Fungal peritonitis in patients on continuous ambulatory peritoneal dialysis. Eur J Clin Microbiol Infect Dis **17**, 839-843 (1998).
18. Gautheret,A., Dromer,F., Bourhis,J.-H., Andremont,A.: *Trichoderma pseudokoningii* as a cause of fatal infection in a bone marrow transplant recipient. Clin Infect Dis **20**, 1063-1064 (1995).
19. Richter,S., Cormican,M.G., Pfaller,M.A., Lee,C.K., Gingrich,R., Rinaldi,M.G., Sutton,D.A.: Fatal disseminated *Trichoderma longibrachiatum* infection in an adult bone marrow transplant patient: Species identification and review of the literature. J Clin Microbiol **37**, 1154-1160 (1999).
20. Jacobs,F., Byl,B., Bourgeois,N., Coremans-Pelseneer,J., Florquin,S., Depré,G., Van de Stadt,J., Adler,M., Gelin,M., Thys,J.P.: *Trichoderma viride* infection in a liver transplant recipient. Mycoses **35**, 301-303 (1992).
21. Furukawa,H., Kusne,S., Sutton,D.A., Manez,R., Carrau,R., Nichols,L., Abu-Elmagd,K., Skedros,D., Todo,S., Rinaldi,M.G.: Acute invasive sinusitis due to *Trichoderma longibrachiatum* in a liver and small bowel transplant recipient. Clin Infect Dis **26**, 487-489 (1998).
22. Guarro,J., Antolin-Ayala,M.I., Gene,J., Gutierrez-Calzada,J., Nieves-Diez,C., Ortoneda,M.: Fatal case of *Trichoderma harzianum* infection in a renal transplant recipient. J Clin Microbiol **37**, 3751-3755 (1999).

23. Munoz,F.M., Demmler,G.J., Travis,W.R., Ogden,A.K., Rossmann,S.N., Rinaldi,M.G.: *Trichoderma longibrachiatum* infection in a pediatric patient with aplastic anemia. J Clin Microbiol **35**, 499-503 (1997).
24. Seguin,P., Degeilh,B., Grulois,I., Gacouin,A., Maugendre,S., Dufour,T., Dupont,B., Camus,C.: Successful treatment of a brain abscess due to *Trichoderma longibrachiatum* after surgical resection. Eur J Clin Microbiol Infect Dis **14**, 445-448 (1995).
25. Hennequin,C., Chouaki,T., Pichon,J.C., Strunski,V., Raccurt,C.: Otitis externa due to *Trichoderma longibrachiatum*. Eur J Clin Microbiol Infect Dis **19**, 641-642 (2000).
26. Samuels,G.J., Petrini,O., Kuhls,K., Lieckfeldt,E., Kubicek,C.P.: The *Hypocrea schweinitzii* complex and *Trichoderma* sect. *Longibrachiatum*. Stud Mycol **41**, 1-54 (1998).
27. Amato,J.G., Amato,V.S., Amato Neto,V., Heins-Vaccari,E.M.: [An AIDS patient with *Trichoderma*]. Rev Soc Brasil Med Tropic **35(3)**, 269 (2002).
28. Escudero,C.M.R., Pino Corral,E., Munoz,R.: Micoma pulmonar causado por *Trichoderma viride*. Actas Dermosifiliogr **67**, 673-680 (1976).
29. Venugopal,P.L., Venugopal,T.L., Gomathi,A., Ramakrishna,E.S., Ilavarasi,S.: Mycotic keratitis in Madras. Indian J Pathol Microbiol **32**, 190-197 (1989).
30. Bustamante-Labarta,M.H., Caramutti,V., Allende,G.N., Weinschelbaum,E., Torino,A.F.: Unsuspected embolic fungal endocarditis of an aortic conduit diagnosed by transesophageal echocardiography. J Am Soc Echocardiogr **13**, 953-954 (2000).
31. Espinel-Ingroff,A.: *In vitro* fungicidal activities of voriconazole, itraconazole, and amphotericin B against opportunistic moniliaceous and dematiaceous fungi. J Clin Microbiol **39**, 954-958 (2001).
32. Espinel-Ingroff,A.: Comparison of the Etest with the NCCLS M38-P method for antifungal susceptibility testing of common and emerging pathogenic filamentous fungi. J Clin Microbiol **39**, 1360-1367 (2001).

33. Robertson,M.H.: Fungi in fluids - a hazard of intravenous therapy. *J Med Microbiol* **3**, 99-102 (1970).
34. Buzina,W., Braun,H., Freudenschuss,K., Lackner,A., Schimpl,H., Stammberger,H.: Biodiversity of fungi in nasal mucus. In: IUMS - The World of Microbes, Xth International Congress of Mycology, Abstracts, EDK Medical and Scientific International Publisher, Paris 2002, p 79
35. Imokawa,S., Sato,A., Taniguchi,M., Imamura,M., Shirai,T., Suda,T., Iwata,M., Gemma,H.: Two cases of acute eosinophilic pneumonia with precipitating antibody against *Trichosporon cutaneum* and *Trichoderma viride*. *Nihon Kyobu Shikkan Gakkai Zasshi (Japan)* **31**, 352-359 (1993).
36. Mouri,M., Nambu,Y., Horii,H., Kobayashi,Y., Yamanouchi,K., Sakurai,S., Toga,H., Ohya,N.: Case report and review of literature on seasonal distribution and pathogenesis of acute eosinophilic pneumonia in Japan. *Nihon Kyobu Shikkan Gakkai Zasshi (Japan)* **31**, 1568-1584 (1993).
37. Larsen,F.O., Clementsen,P., Hansen,M., Maltbaek,N., Gravensen,S., Stahl Skov,P., Norn,S.: The indoor microfungus *Trichoderma viride* potentiates histamine release from human bronchoalveolar cells. *APMIS* **104**, 673-679 (1996).
38. Antal,Z., Kredics,L., Dóczy,I., Manczinger,L., Kevei,F., Nagy,E.: The physiological features of opportunistic *Trichoderma* strains. *Acta Microbiol Immunol Hung* **49**, 393 (2002).
39. Rinaldi,M.G., Howell,A.W.: Antifungal antimicrobics: laboratory evaluation. In: Wentworth,B. (ed): *Diagnostic procedures for mycotic and parasitic infections*. 7th ed. American Public Health Association, Washington, D.C. 1988, pp 325-356
40. Etest technical guide 10: Antifungal susceptibility testing of moulds. AB Biodisk, Solna, Sweden (1999)

41. National Committee for Clinical Laboratory Standards. Reference method for broth dilution antifungal susceptibility testing of conidium forming filamentous fungi: Proposed standard M38-P. National Committee for Clinical Laboratory Standards, Wayne, Pa. 1998
42. Pujol,I, Guarro,J., Llop,C., Soler,L., Fernández-Ballart,J.: Comparison study of broth macrodilution and microdilution antifungal susceptibility tests for the filamentous fungi. *Antimicrob Agents Chemother* **40**, 2106-2110 (1996).
43. Shadomy,S., Pfaller,M.: Laboratory studies with antifungal agents: susceptibility tests and quantitation in body fluids. In: Balows,A. et al. (ed): *Manual of clinical microbiology*, 5th ed. American Society for Microbiology, Washington, D.C. 1991, pp 1173-1183
44. Drouhet,E., Dupont,B., Improvisi,L., Viviani,M.A., Tortorano,A.M.: Disc agar diffusion and microplate automatized technics for *in vitro* evaluation of antifungal agents on yeasts and sporulated pathogenic fungi. In: Iwata,K., Vanden Bossche,H. (eds): *In vitro and in vivo* evaluation of antifungal agents. Elsevier Science Press, Paris 1986, pp 31-49
45. Naár,Z., Kecskés,M.: Factors influencing the competitive saprophytic ability of *Trichoderma* species. *Microbiol Res* **153**, 119-129 (1998).
46. Antal,Z., Manczinger,L., Szakács,G., Tengerdy,R.P., Ferenczy,L.: Colony growth, *in vitro* antagonism and secretion of extracellular enzymes in cold-tolerant strains of *Trichoderma* species. *Mycol Res* **104**, 545-549 (2000).
47. Kredics,L., Antal,Z., Manczinger,L.: Influence of water potential on growth, enzyme secretion and *in vitro* enzyme activities of *Trichoderma harzianum* at different temperatures. *Curr Microbiol* **40**, 310-314 (2000).
48. Kredics,L., Dóczy,I., Antal,Z., Manczinger,L.: Effect of heavy metals on growth and extracellular enzyme activities of mycoparasitic *Trichoderma* strains. *Bull Environ Contam Toxicol* **66**, 249-254 (2001).

49. Manczinger,L., Molnár,A., Kredics,L., Antal,Z.: Production of bacteriolytic enzymes by mycoparasitic *Trichoderma* strains. World J Microbiol Biotechnol **18**, 147-150 (2002).
50. Goldfarb,B., Nelson,E.E., Hansen,E.M.: *Trichoderma* spp.: Growth rates and antagonism to *Phellinus weirii* in vitro. Mycologia **81**, 375-381 (1989).
51. Widden,P.: The effects of temperature on competition for spruce needles among sympatric species of *Trichoderma*. Mycologia **76**, 873-883 (1984).
52. Kredics,L., Antal,Z., Manczinger,L., Kevei,F., Nagy,E.: Proteolytic enzyme profiles of clinical *Trichoderma* isolates. Acta Microbiol Immunol Hung **49**, 416-417 (2002).
53. Kuhls,K., Lieckfeldt,E., Börner,T., Guého,E.: Molecular reidentification of human pathogenic *Trichoderma* isolates as *Trichoderma longibrachiatum* and *Trichoderma citrinoviride*. Med Mycol **37**, 25-33 (1999).
54. Hogan,L.H., Klein,B.S., Levitz,S.M.: Virulence factors of medically important fungi. Clin Microbiol Rev **9**, 469-488 (1996).

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Table ICase reports about *Trichoderma* infections in the literature

Age/Sex	Clinical diagnosis	Source	Etiology	Therapy	Outcome	Reference
82/M	CAPD peritonitis	Peritoneal fluid	<i>T. harzianum</i>	K, 5FC	Death	[10]
63/F	CAPD peritonitis	Peritoneal fluid	<i>T. koningii</i>	Catheter removal, M	Survival	[11]
41/M	CAPD peritonitis	Peritoneal fluid	<i>T. koningii</i>	F, 5FC, AB	Death	[12]
48/M	CAPD peritonitis	Peritoneal fluid, autopsy	<i>T. longibrachiatum</i>	AB	Death	[13]
33/M	APD peritonitis	Peritoneal fluid	<i>T. pseudokoningii</i>	Catheter removal	Survival	[14]
47/M	CAPD peritonitis	Peritoneal fluid, autopsy	<i>T. viride</i>	AB	Death	[15]
44/M	CAPD peritonitis	Peritoneal fluid	<i>T. viride</i>	AB	Death	[16]
60/M	CAPD peritonitis	Peritoneal fluid	<i>Trichoderma sp.</i>	Catheter removal, K	Survival	[17]
45/F	TX/Lung and skin dissemination	Bronchoalveolar lavage, skin biopsy	<i>T. pseudokoningii</i>	F, AB, 5FC	Death	[18]
29/M	TX/Disseminated infection	Lung, liver, intestinal wall, autopsy	<i>T. longibrachiatum</i>	AB, I, liposomal AB	Death	[19]
44/F	TX/Abdominal dissemination	Peritoneal fluid, haematoma	<i>T. viride</i>	Surgery, AB, F	Death	[20]
29/F	TX/Acute invasive sinusitis	Sinus debridement	<i>T. longibrachiatum</i>	Surgery, AB, I	Survival	[21]
68/M	TX/Disseminated infection	Brain and lung abscesses, autopsy	<i>T. harzianum</i>	-	Death	[22]
11/M	Skin infection	Skin biopsy	<i>T. longibrachiatum</i>	AB	Survival	[23]
17/F	Brain abscess	Brain biopsy, cerebral pus	<i>T. longibrachiatum</i>	Surgery, AB, 5FC, K, I	Survival	[24]
12/ND	Otitis externa	Ear discharge	<i>T. longibrachiatum</i>	nystatin, polymyxin B	Survival	[25]
46/M	Pulmonary mycetoma	Sputum, lung biopsy	<i>T. viride</i>	Surgery	ND	[28]
66/M	Endocarditis	Aortic conduit	<i>Trichoderma sp.</i>	Surgery, antifungal drugs	Survival	[30]
26/F	Fungemia by contaminated saline	Blood	<i>Trichoderma viride</i>	AB	Survival	[33]

M = male, F = female; APD = automated peritoneal dialysis, CAPD = chronic ambulatory peritoneal dialysis; 5FC = 5-fluorocytosine, AB = amphotericin B, F = fluconazole, I = itraconazole, K = ketoconazole, M = miconazole; TX = transplant; ND = no data available

Table IIAntifungal susceptibilities of clinical *Trichoderma* isolates

Isolate	Method	Antifungal agent	MIC ($\mu\text{g/ml}$)	Reference
<i>T. longibrachiatum</i> UAMH 7955	Macrobroth dilution [39]	5FC	>322.75	[21]
		AB	1.16	
		F	80	
		I	0.3	
	Etest [40]	AB	1	[38]
		F	>256	
		I	32	
Disk method	K	0.125		
V	*7 mm			
<i>T. longibrachiatum</i> UAMH 7956	NCCLS M-38P [41]	5FC	>256	[19]
		AB	2.0	
		F	16	
		I	1.0	
	Etest [40]	AB	2	[38]
		F	64	
		I	16	
Disk method	K	0.25		
V	*9 mm			
<i>T. longibrachiatum</i> UAMH 9515	Etest [40]	AB	2	[38]
		F	>256	
		I	16	
	Disk method	K	0.5	
		V	*9 mm	
<i>T. longibrachiatum</i> ATCC 201044	NCCLS broth macrodilution	5FC	>64	[23]
		AB	2	
		F	>64	
		I	2	
	Etest [40]	AB	2	[38]
		F	64	
		I	0.5	
K	0.008			
<i>T. longibrachiatum</i> ATCC 208859	Etest [40]	AB	2	[38]
		F	>256	
		I	8	
		K	0.25	
<i>T. longibrachiatum</i> IP-93 1192	Broth dilution	5FC	50	[24]
		AB	2.5	
		F	12.5	
		I	1.25	
	Disk method	K	*34 mm	
<i>T. longibrachiatum</i>	Broth dilution	AB	5	[13]
<i>T. longibrachiatum</i>	Etest	AB	24	[25]
		I	32	
<i>T. longibrachiatum</i> (5 isolates)	NCCLS M-38P [41]	AB	0.5-2	[31]
		I	>8	
		V	2	
	Etest	AB	1.0-4	[32]
		I	>8	

Table II (continued)

Isolate	Method	Antifungal agent	MIC ($\mu\text{g/ml}$)	Reference
<i>T. citrinoviride</i> UAMH 9573	Etest [40]	AB	2	[38]
		F	64	
	I	32		
	K	0.25		
	Disk method	V	*9 mm	
<i>T. harzianum</i> CBS 102174	Microdilution [42]	5FC	256	[22]
		AB	2	
		F	128	
		I	32	
		K	8	
		M	8	
<i>T. koningii</i> CM 382	Broth microdilution [43]	5FC	16.0	[12]
		AB	4.0	
		F	128.0	
		K	1.0	
		M	≤ 0.25	
	Etest [40]	AB	8	[38]
		F	>256	
		I	2	
	Disk method	K	0.25	
		V	*8 mm	
<i>T. koningii</i>	ND	5FC	ND, resistant	[11]
		AB	ND, resistant	
		K	ND, susceptible	
		M	ND, susceptible	
<i>T. pseudokoningii</i> IP-92 2110	Broth microdilution [44]	5FC	>100	[18]
		AB	0.09	
		F	25	
		I	0.18	
		M	0.09	
<i>T. viride</i>	Broth dilution	5FC	100	[15]
		AB	0.78	
		K	1.56	
<i>T. viride</i>	Broth dilution	5FC	>50	[20]
		AB	3.1	
		F	25	
		I	1.6	
		K	0.8	

5FC = 5-fluorocytosine, AB = amphotericin B, F = fluconazole, I = itraconazole,

K = ketoconazole, M = miconazole, V = voriconazole

ND = no specific data, * = diameter of inhibition zone

ATCC = American Type Culture Collection

CBS = Centraalbureau voor Schimmelcultures

CM = Centro Nacional de Microbiologia, Immunologia y Virologia Sanitaria

IP = Institut Pasteur

UAMH = University of Alberta Microfungus Collection and Herbarium