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CLINICAL IMPORTANCE OF THE GENUS *TRICHODERMA*

A REVIEW*

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Opportunistic fungal infections have been observed 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 in immunocompromised hosts as well. This review is going to summarize the clinical case reports about *Trichoderma* infections, and to discuss the information available on the antifungal susceptibility and on the ecophysiological, enzymological and systematic aspects of clinical *Trichoderma* isolates.

Keywords: *Trichoderma*, opportunistic fungi, antifungal susceptibility testing, ecophysiology, extracellular proteases, species identification

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 participate in the decomposition of plant residues in the soil. Some *Trichoderma* species are very good cellulase producers

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

Trichoderma infection case reports 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 each of these cases. Three patients survived [11, 14, 17]. The early catheter removal proved to be a succesfull 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 occured 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

Table I

Trichoderma infection case reports in the literature

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

caused by *T. longibrachiatum* in a liver and small bowel transplant recipient was succesfully treated with surgical debridements and amphotericin B followed by oral itraconazole [21]. Guarro et al. detected a systemic *T. harzianum* infection in a post mortem 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 from 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 inadver-

tent 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 person 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. In Table II data available about the susceptibilities of clinical *Trichoderma* isolates – including our Etest results [38] about the susceptibilities of strains *T. citrinoviride* UAMH 9573, *T. koningii* CM 382 ([12], kindly provided by the Centro Nacional de Microbiologia, Immunologia y Virologia Sanitaria, Mycology Section, Majadahonda, Madrid), *T. longibrachiatum* ATCC 201044 [23], ATCC 208859, UAMH 7955 [21], UAMH 7956 [19] and UAMH 9515. 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 [11–13, 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 treatment of infected patiens. In cases of suspected or confirmed *Trichoderma* infection, amphotericin B alone or in combination with itra-

Table II

Antifungal susceptibilities of clinical *Trichoderma* isolates

Isolate	Method	Antifungal agent	MIC (μg/ml)	Reference
T. longibrachiatum 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	
		K	0.125	
	Disk method	V	*7 mm	
T. longibrachiatum 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	
		K	0.25	
	Disk method	V	*9 mm	
T. longibrachiatum UAMH 9515	Etest [40]	AB	2	[38]
		F	>256	
		I	16	
		K	0.5	
	Disk method	V	*9 mm	
T. longibrachiatum 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	
T. longibrachiatum ATCC 208859	Etest [40]	AB	2	[38]
		F	>256	
		I	8	
		K	0.25	
T. longibrachiatum IP-93 1192	Broth dilution	5FC	50	[24]
		AB	2.5	
		F	12.5	
		I	1.25	
	Disk method	K	*34 mm	
T. longibrachiatum	Broth dilution	AB	5	[13]
T. longibrachiatum	Etest	AB	24	[25]
		I	32	
T. longibrachiatum (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 cont.)

Isolate	Method	Antifungal agent	MIC (μg/ml)	Reference
T. citrinoviride UAMH 9573	Etest [40]	AB	2	[38]
		F	64	
		I	32	
		K	0.25	
	Disk method	V	*9 mm	
T. harzianum CBS 102174	Microdilution [42]	5FC	256	[22]
		AB	2	
		F	128	
		I	32	
		K	8	
		M	8	
T. koningii 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	
		K	0.25	
	Disk method	V	*8 mm	
T. koningii	ND	5FC	ND, resistant	[11]
		AB	ND, resistant	
		K	ND, susceptible	
		M	ND, susceptible	
T. pseudokoningii IP-92 2110	Broth microdilution [44]	5FC	>100	[18]
		AB	0.09	
		F	25	
		I	0.18	
		M	0.09	
T. viride	Broth dilution	5FC	100	[15]
		AB	0.78	
		K	1.56	
T. viride	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

conazole 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 the 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], there is, however, a vacancy 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 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* have shown, 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 belong the Longibrachiatum section of the genus. The pH ranging from 2.0 to 9.0 supported the growth of most of the strains examined at 25 °C, with an optimum at pH 4.0. 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 compounds, 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 strains examined. However, strain *T. koningii* CM 382 did not produce 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 cases are reported from patients undergoing peritoneal dialysis and from transplant recipients. *T. longibrachiatum* is the most frequently occuring etiologic agent within the genus. The detailed case reports and data about the antifungal susceptibilities of clinical *Trichoderma* isolates yield 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 a 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 on the basis of 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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