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Characterization of Clinical Isolates of *Talaromyces marneffei* and Related Species, California, USA

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Talaromyces marneffei and other *Talaromyces* species can cause opportunistic invasive fungal infections. We characterized clinical *Talaromyces* isolates from patients in California, USA, a non–*Talaromyces*-endemic area, by a multiphasic approach, including multigene phylogeny, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, and phenotypic methods. We identified 10 potentially pathogenic *Talaromyces* isolates, 2 *T. marneffei*.

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Talaromyces marneffei is a dimorphic fungal pathogen that causes focal or systemic infection in immunocompromised persons, primarily HIV-infected patients (*1*). Many cases have been reported in travelers returning from areas of Southeast Asia, southern China, and eastern India to which it is endemic. Other *Talaromyces* species also have been reported to cause invasive fungal infections, including *T. amestolkiae* (*2*), *T. purpurogenus* (*3*,*4*), and *T. piceus* (*5*,*6*). *Talaromyces* species are common in air, soil, and human habitats. Clinical laboratories in areas to which this fungus is not endemic often do not perform identification of *T. marneffei* and other *Talaromyces* species (*2*). Therefore, we devised a multiphasic approach for identifying *T. marneffei* and other potentially pathogenic *Talaromyces* species.

We conducted this study during 2018. *Talaromyces* isolates from 10 human specimens were submitted to the Microbial Diseases Laboratory (MDL), California Department of Public Health (Richmond, CA, USA), to rule out *T. marneffei* (Appendix, https://wwwnc.cdc.gov/EID/article/25/9/19-0380-App1.pdf). Temperature and pH are known to influence pigment production and colony morphology of *Talaromyces* species; therefore, growth characteristics were observed using 2 different culture media (Sabouraud dextrose agar, pH 5.6; and Sabouraud dextrose agar, Emmons, pH 6.9), incubated at 25°C and 30°C. Fungal DNA was extracted using a previously reported method (*7*). *Talaromyces* isolates were identified to species level using the internal transcribed spacer (ITS) region, partial β-tubulin gene (BenA), and partial RNA polymerase II largest subunit gene (RPB1) (*8*). The ITS and partial BenA and RPB1 sequences were used to search for homologies in GenBank and CBS databases (http://www.westerdijkinstitute.nl/collections). Multigene phylogenetic analysis was conducted on the concatenated ITS–BenA–RPB1 nucleotide sequence alignment (Appendix). A blastn search (https://blast.ncbi.nlm.nih.gov/blast) through the GenBank database, pairwise comparison alignment through the CBS database, or both showed 99%–100% homology for ITS, 97%–100% for BenA, and 91%–100% for RPB1 sequences with the best-matched sequences of known *Talaromyces* species isolates.

Phylogenetic analysis of the *Talaromyces* isolates showed 7 genetic clades, consistent with previous descriptions of the *Talaromyces* genera (*9*) (Figure). Species identification using a comparison of the ITS, BenA, and RPB1 sequences with existing sequences and multigene phylogenetic analysis identified *T. marneffei* (isolates MDL17022 and MDL18026), *T. atroroseus* (MDL17026, MDL17144, MDL17164, and MDL18070), *T. islandicus* (MDL18167), *T. stollii* (MDL18054), *T. coalescens* (MDL18102), and *T. australis* (MDL18159). The 2 *T. marneffei* isolates produced diffuse red pigment early, by 3 days of growth, on both medium types and at both incubation temperatures. *T. australis* and *T. stollii* isolates also produced red pigment by 3 days but with variations based on media or temperature. At 7 days of growth, the 4 *T. atroroseus* isolates also showed variable red pigment production (abundant, weak, and absent) (Appendix). Microscopically, most isolates showed biverticillate conidiophores and globose to fusiform conidia in unbranched chains. Both *T. marneffei* isolates were from HIV-positive patients. MDL17022 was from a blood sample of a 37-year-old man with a travel history to Southeast Asia; MDL18026 was from skin tissue of a 36-year-old man with no available travel history.

Using matrix-assisted laser desorption/ionization-time-of-flight (MALDI-TOF) mass spectrometry, we generated main spectrum profiles (MSP) of *Talaromyces* species following Bruker's custom MSP and library creation standard operating procedure (https://www.bruker.com). We extracted proteins of *Talaromyces* isolates using the previously published National Institutes of Health (NIH) protocol (*10*). We analyzed *Talaromyces* spectra with MALDI Biotyper 4.1 software against combined databases of the Filamentous Fungi Library 2.0 (Bruker) and the NIH Mold Library (*10*), with and without inclusion of newly created MSPs of *Talaromyces* species (Appendix). The threshold for species identification was >1.9; for genus identification, ≥1.7.

Using the combined databases of Filamentous Fungi Library 2.0 (Bruker) and NIH Mold Library, we identified none of the isolates to species level; results showed either no identification or genus-level identification. However, when we expanded the combined database with the MDL Mold Library, we correctly identified all *Talaromyces* isolates to the species level with the best score ≥1.9. There were no ambiguous identification results; that is, the second-best matched species also had a high confidence score ≥1.9.

T. marneffei can be readily differentiated from other red pigment–producing *Talaromyces* species by yeast-like colony conversion at 37°C. However, many clinical laboratories no longer conduct yeast conversions. For those laboratories, yellow-green colonies producing red soluble pigment at ≈3 days on common fungal culture media at 25°C–30°C might indicate the need to further confirm *T. marneffei*. It is difficult to distinguish *Talaromyces* species only by macroscopic and microscopic examination. Multilocus sequencing, although confirmatory, might be too time-consuming and expensive for routine use. Therefore, we identified all *Talaromyces* isolates to species level by MALDI-TOF mass spectrometry by using an expanded database with well-characterized *Talaromyces* strains.

In conclusion, our results show that MALDI-TOF mass spectrometry is a good choice for rapid, less expensive primary identification of *Talaromyces* species and other medically important fungal pathogens. Species-level

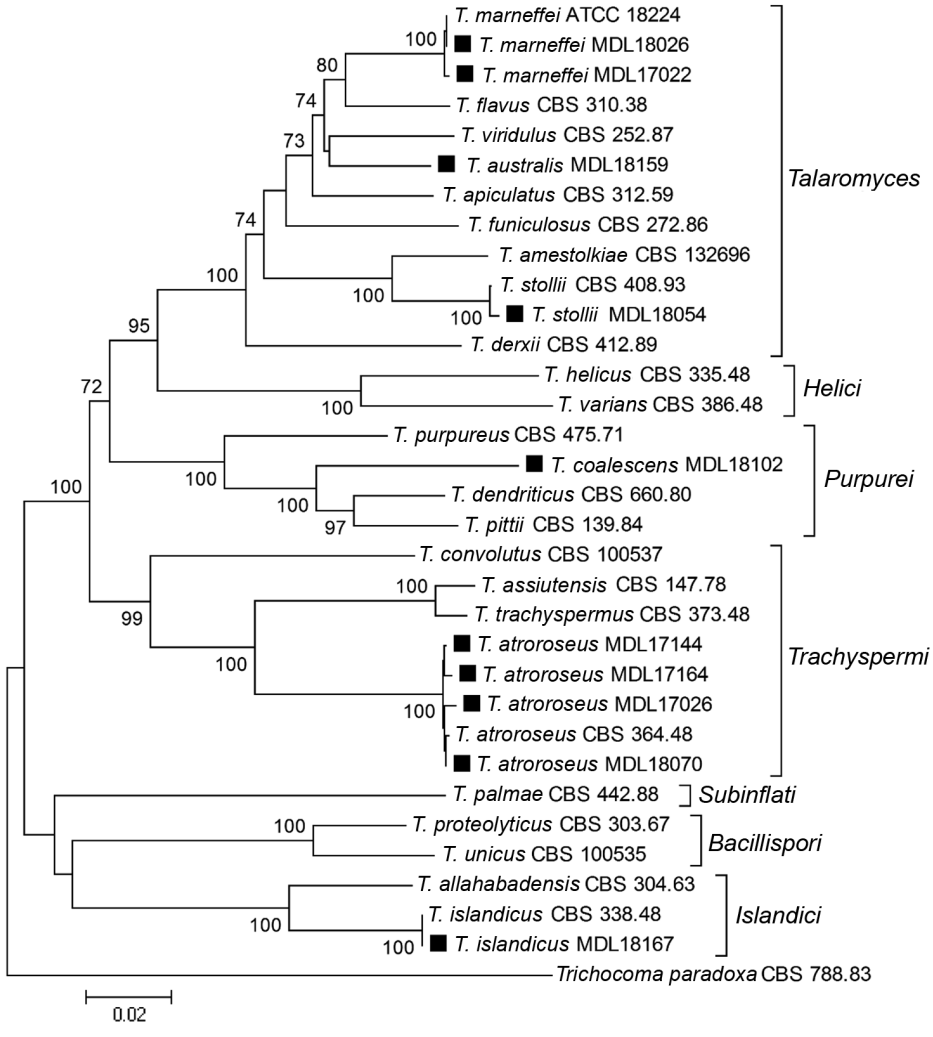

Figure. Phylogenetic analysis of *Talaromyces* species based on concatenated nucleotide alignments of internal transcribed spacer, partial β-tubulin gene, and partial RNA polymerase II largest subunit gene regions, showing the relationship among clinical isolates from patients in California, USA (black squares), and reference *Talaromyces* species. The tree was constructed by the neighbor-joining method with 1,000 bootstrap replicates by using MEGA software (https://www.megasoftware.net). Bootstrap support values >70% are presented at the nodes. The tree was rooted with *Trichocoma paradoxa* CBS 788.83. GenBank accession numbers for newly generated sequences are MK601832–41 for the internal transcribed spacer, MK626499–508 for the β-tubulin gene, and MK626509–518 for the RNA polymerase II largest subunit gene. CBS, Westerdijk Fungal Biodiversity Institute; MDL, Microbial Diseases Laboratory, California Department of Public Health. Scale bar indicates estimated phylogenetic divergence.

identification of *Talaromyces* isolates is clinically useful for treatment of patients with underlying conditions, such as immunodeficiency, cancer, advanced age, and immunosuppressive therapy.

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Parathyridaria percutanea and Subcutaneous Phaeohyphomycosis

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Parathyridaria percutanea is an emerging fungus causing subcutaneous phaeohyphomycoses in renal transplant recipients in India. We identified *P. percutanea* from a patient with subcutaneous phaeohyphomycosis. From our culture collection, we identified the same fungus from 4 similar patients. We found 5 cases previously described in literature.

[1]These first authors contributed equally to this article.

Parathyridaria percutanea, earlier known as *Roussoella percutanea* in the order *Pleosporales,* has been reported to cause subcutaneous phaeohyphomycoses (*1*,*2*). *P. percutanea* belongs to coelomycetes, a group of fungi in which the conidia or asexual propagules lie within a cavity. *Parathyridaria* spp. generally exist as plant saprobes; *P. percutanea* is the only species reported as an opportunistic pathogen.

We recently observed a case of subcutaneous phaeohyphomycosis caused by *P. percutanea.* The patient was a 33-year-old man who had ACTH-dependent Cushing's disease with 2 cutaneous lesions, one under the left axilla and the other on the ulnar aspect of the left forearm, that had progressed slowly over 3 years (Appendix Figure 1, panel A, https://wwwnc.cdc.gov/EID/article/25/9/19-0383-App1.pdf). Direct microscopy of a biopsy sample taken from the left forearm lesion revealed dematiaceous septate hyphae with irregular hyphal swellings (Appendix Figure 1, panel B). Colonies on Sabouraud's dextrose agar at 25°C were flat, spreading with sparse aerial hyphae after 1 week, and later turned to cottony greenish-black growth (Appendix Figure 1, panel C). Lactophenol cotton blue mount revealed nonsporulating dematiaceous hyphae with chlamydospores (Appendix Figure 1, panel D). Several attempts to induce sporulation (on oatmeal agar and malt extract agar) failed. Histopathologic examination (Appendix Figure 1, panels E–G) showed neutrophilic infiltration with fungal hyphae, nodular swellings on Giemsa stain, and black hyphae on Grocott-Gomori's methamine silver stain.

We identified the fungus as *Roussoella percutanea* of the order *Pleosporales,* later renamed *P. percutanea,* by PCR sequencing of the internal transcribed spacer (ITS) and 28S regions of ribosomal DNA, as described previously (*3*). ITS sequencing of our strain NCCPF104001 (GenBank accession nos. MG708109 [by ITS] and MG708116 [by 28S]) had 99.8% identity with CBS128203 (type strain, GenBank accession no. KF322117) and CBS868.95 (GenBank accession no. KF322118), whereas 28S sequences had 100% identity with CBS128203 (GenBank accession no. KF366448) and CBS868.95 (GenBank accession no. KF366449) (Appendix Figure 2, panels A. B). The patient refused further treatment in the hospital and left against medical advice.

We screened all the isolates deposited in our National Culture Collection of Pathogenic Fungi (NCCPF, Chandigarh) and characterized them phenotypically as *Pleosporales*. Of 7 such isolates, we identified 4 as *P. percutanea* by sequencing (Table, https://wwwnc.cdc.gov/EID/article/25/9/19-0383-T1.htm). We further subjected these isolates to phylogenetic analysis of ITS and large ribosomal subunit (28S) of the rDNA using MEGA software version 6 (https://megasoftware.net) (*3*). The strains identified as *P. percutanea* clustered together with the ITS and 28S sequences of CBS12608 and CBS868.95 strains, the other 2 *P. percutanea*

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Clinical Isolates of *Talaromyces marneffei* and Related Species, California, USA

Appendix

Appendix Table 1. Source of *Talaromyces* isolates

Isolate ID	Culture source	Age/sex	Species
MDL17022	Blood sample	37/M	*T. marneffei*
MDL17026	Sputum sample	53 /F	*T. atroroseus*
MDL17144	Ear	9 /M	*T. atroroseus*
MDL17164	Nail	85/M	*T. atroroseus*
MDL18026	Skin tissue	36/M	*T. marneffei*
MDL18054	Sputum	52/F	*T. stollii*
MDL18070	Bronchial wash	73/F	*T. atroroseus*
MDL18102	Bronchoalveolar lavage sample	59/F	*T. coalescens*
MDL18159	Bronchoscopy sample	72/F	*T. australis*
MDL18167	Bronchial wash	63/M	*T. islandicus*

Appendix Table 2. Identification of *Talaromyces* isolates by matrix-assisted laser desorption/ionization–time of flight mass spectrometry*

		Bruker and NIH database†		Bruker, NIH, and MDL database	
Isolate	Species ID by sequencing	Best match	Best score	Best match	Best score
MDL17022	*T. marneffei*	*T. marneffei*	1.22	*T. marneffei*	2.31
MDL17026	*T. atroroseus*	No ID	–	*T. atroroseus*	2.18
MDL17144	*T. atroroseus*	No ID	–	*T. atroroseus*	2.30
MDL17164	*T. atroroseus*	No ID	–	*T. atroroseus*	2.57
MDL18026	*T. marneffei*	No ID	–	*T. marneffei*	2.17
MDL18054	*T. stollii*	*T. ruber*	1.78	*T. stollii*	2.36
MDL18070	*T. atroroseus*	No ID	–	*T. atroroseus*	2.23
MDL18102	*T. coalescens*	No ID	–	*T. coalescens*	2.02
MDL18159	*T. australis*	*Talaromyces spp.*	1.38	*T. australis*	2.31
MDL18167	*T. islandicus*	No ID	–	*T. islandicus*	2.02

*ID, identification; MDL, Microbial Diseases Laboratory, California Department of Public Health; NIH, National Institutes of Health. Dash indicates . †Bruker Filamentous Fungi Library 2.0 and NIH Mold Library (Lau AF, Drake SK, Calhoun LB, Henderson CM, Zelazny AM. Development of a clinically comprehensive database and a simple procedure for identification of molds from solid media by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol. 2013;51:828–34).

Appendix Table 3. Growth study of *Talaromyces* isolates*

					Red soluble pigment†							
					3 d				7 d			
		37°C growth	Colony characteristics on SDA 30°C		SDA		E-SDA		SDA		E-SDA	
Isolate ID	Species ID	on SDA	3 d	7 d	25	30	25	30	25	30	25	30
MDL17022	*T. marneffei*	+	Yellowish white, velvety	Yellowish green, reddish brown, floccose	+	+	+	+	+	+	+	+
MDL17026	*T. atroroseus*	+	Blue green, felty	Blue green, floccose, white center	–	–	–	–	–	–	–	–
MDL17144	*T. atroroseus*	+	White, downy	Dark blue green, velvety, red center	–	–	–	–	–	+	+	+
MDL17164	*T. atroroseus*	+	White, downy	Dark blue green, floccose, yellow center	–	–	–	–	–	+	–	–
MDL18026	*T. marneffei*	+	Yellowish green, powdery	Yellowish green, pinkish brown, floccose	+	+	+	+	+	+	+	+
MDL18054	*T. stollii*	+	White, floccose	White, floccose	–	–	+	–	+	–	+	–
MDL18070	*T. atroroseus*	+	White, downy	Blue green, reddish brown, floccose	–	–	–	–	+	+	+	–
MDL18102	*T. coalescens*	–	Whitish green, downy	Whitish-green, floccose	–	–	–	–	–	–	–	–

Isolate ID	Species ID	37°C growth on SDA	Colony characteristics on SDA 30°C		Red soluble pigment†							
					3 d				7 d			
					SDA		E-SDA		SDA		E-SDA	
			3 d	7 d	25	30	25	30	25	30	25	30
MDL18159	*T. australis*	+	White, red, floccose	Red, floccose	–	+	+	+	+	+	+	+
MDL18167	*T. islandicus*	+	Green, orange, felty	Dark green, felty, orange edge	–	–	–	–	–‡	–‡	–‡	–‡

*E-SDA, Sabouraud dextrose agar, Emmons; SDA, Sabouraud dextrose agar; +, positive ; –, negative.
†Red soluble pigment producing was observed at 25°C and 30°C.
‡*T. islandicus* produces yellow-orange soluble pigment.

Appendix Table 4. *Talaromyces* isolate reference sequences and their GenBank accession numbers*

Species	Collection no.	ITS	BenA	RPB1
T. allahabadensis	CBS 304.63	KF984873	KF984614	JN680309
T. amestolkiae	CBS 132696	JX315660	JX315623	JX315679
T. apiculatus	CBS 312.59	JN899375	KF741916	JN680293
T. assiutensis	CBS 147.78	JN899323	KJ865720	JN680275
T. atroroseus	CBS 364.48	KF114740	KF114790	KF114750
T. convolutus	CBS 100537	JN899330	KF114773	JN121553
T. dendriticus	CBS 660.80	JN899339	JX091391	JN121714
T. derxii	CBS 412.89	JN899327	JX494306	JN680306
T. flavus	CBS 310.38	JN899360	JX494302	JN121639
T. funiculosus	CBS 272.86	JN899377	JX091383	JN680288
T. helicus	CBS 335.48	JN899359	KJ865725	JN680300
T. islandicus	CBS 338.48	KF984885	KF984655	JN121648
T. marneffei	ATCC 18224	NW_002196683	NW_002196666	NW_002196662
T. palmae	CBS 442.88	JN899396	HQ156947	JN680308
T. pittii	CBS 139.84	JN899325	KJ865728	JN680274
T. proteolyticus	CBS 303.67	JN899387	KJ865729	JN680292
T. purpureus	CBS 475.71	JN899328	GU385739	JN121687
T. stollii	CBS 408.93	JX315674	JX315633	JX315693
T. trachyspermus	CBS 373.48	JN899354	KF114803	JN121664
T. unicus	CBS 100535	JN899336	KJ865735	JN680324
T. varians	CBS 386.48	JN899368	KJ865731	JN680305
T. viridulus	CBS 252.87	JN899314	JX091385	JN680284
Trichocoma paradoxa	CBS 788.83	JN899398	KF984556	JN121718

*ATCC, American Type Culture Collection; BenA: β-tubulin gene; CBS, Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands; ITS, internal transcribed spacer; RPB1, RNA polymerase II largest subunit gene.