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About the Author

Dr. Hamprecht is a clinical microbiologist at the Institute for Medical Microbiology, Immunology and Hygiene and professor for antibiotic resistance of gram-negative pathogens at the University of Cologne, Germany, and the German Centre for Infection Research (DZIF), also in Cologne. His research interests include multidrug-resistant organisms (mainly Enterobacterales and fungi), their resistance mechanisms, and the improvement of diagnostic methods.

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Address for correspondence: Oliver Kurzai, University of Würzburg Institute for Hygiene and Microbiology, Josef-Schneider-Straße 2 / E1, Würzburg 97080, Germany; email: okurzai@hygiene.uni-wuerzburg.de

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

Linlin Li, Katelyn Chen, Nirmala Dhungana, Yvonne Jang, Vishnu Chaturvedi,[1] Ed Desmond[2]

Author affiliation: California Department of Public Health, Richmond, 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*.

[1]Current affiliation: New York State Department of Health, Albany, New York, USA.

[2]Current affiliation: Hawaii State Department of Health, Pearl City, Hawaii, USA.

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

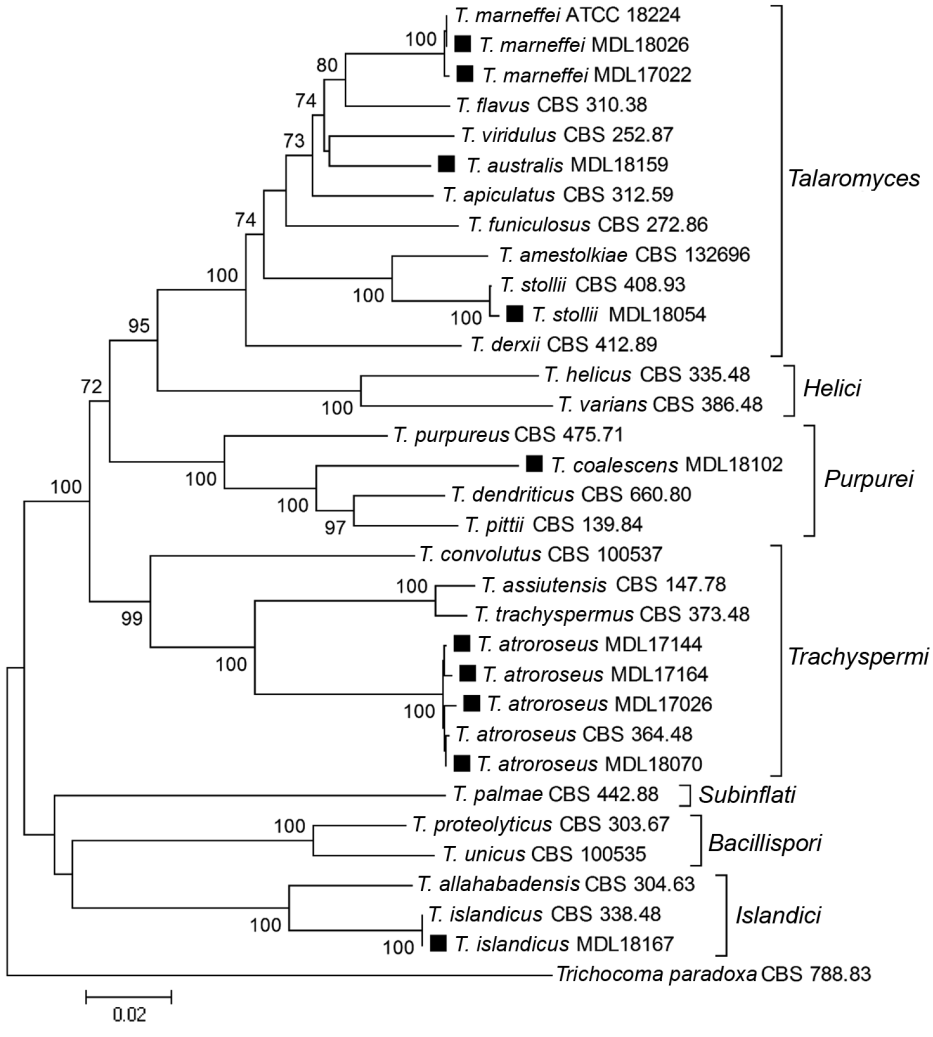

Figure. Phylogenetic analysis of *Talaromyces* species based on concatenated nucleotide alignments of internal transcribed spacer, partial β-tubulin gene, and partial RNA polymerase II largest subunit gene regions, showing the relationship among clinical isolates from patients in California, USA (black 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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About the Author

Dr. Li is a research scientist and a certified public health microbiologist at the Microbial Diseases Laboratory, California Department of Public Health. Her primary research interests include molecular diagnosis of mycotic diseases, Valley fever, candidemia, antifungal susceptibility testing, mycobacteriology, metagenomics, and next-generation sequencing.

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Address for correspondence: Ed Desmond, State Laboratories Division, Hawaii State Department of Health, 2725 Waimano Home Rd, Pearl City, HI 96782, USA; email: edward.desmond@doh.hawaii.gov

Parathyridaria percutanea and Subcutaneous Phaeohyphomycosis

Shivaprakash M. Rudramurthy,[1] Megha Sharma,[1] Nandini Sethuraman, Pinaki Dutta, Bansidhar Tarai, Jayanthi Savio, Amanjit Bal, Usha Kalawat, Arunaloke Chakrabarti

Author affiliations: Postgraduate Institute of Medical Education and Research, Chandigarh, India (S.M. Rudramurthy, M. Sharma, N. Sethuraman, P. Dutta, A. Bal, A. Chakrabarti); Apollo Hospitals, Chennai, India (N. Sethuraman); Max Super Speciality Hospitals, New Delhi, India (B. Tarai); St. Johns Medical College and Research Institute, Bengaluru, India (J. Savio); Sri Venkateshwara Institute of Medical Sciences, Tirupati, India (U. Kalawat)

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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*