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Three-Locus Identification, Genotyping, and Antifungal Susceptibilities of Medically Important *Trichosporon* Species from China▽‡

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Three reference and 45 clinical isolates of *Trichosporon* were analyzed by conventional phenotypic and molecular methods to determine the species and genotypes of *Trichosporon* isolates from China. Target loci for molecular methods included the internal transcribed spacer (ITS) region, the D1/D2 domain of the 26S rRNA gene, and the intergenic spacer 1 (IGS1) region. Identification of eight *Trichosporon* species was achieved, of which *Trichosporon asahii* was the most common. Of the sequence-based molecular methods, the one targeting the D1/D2 domain assigned 97.9% (47/48) of isolates (seven species) correctly, while tests targeting both the ITS and IGS1 regions correctly identified all 48 isolates. The commercial API 20C AUX and Vitek 2 Compact YST systems correctly identified 91.9% and 73% of isolates when their biochemical profiles were queried against those of species contained in the databases, respectively, and misidentified 63.6% and 36.4% of isolates of species that were unclaimed by the databases, respectively. The predominant genotype among *T. asahii* clinical isolates, genotype 4 (51.4%), is rarely found in other countries. Voriconazole and itraconazole were the most active drugs *in vitro* against all the *Trichosporon* species tested, while caspofungin and amphotericin B demonstrated poor activity.

Trichosporon species are emerging fungal pathogens capable of causing localized or systemic mycoses. Disseminated infection typically occurs in patients with underlying hematological malignancy, those who have undergone organ transplantation, and those who are otherwise heavily immunocompromised (5, 9, 18). Such infection is often lethal (mortality rates, 42 to 80%) (4, 13, 27). Furthermore, morbidity is significant, with multiple sequelae, including respiratory and renal failure and disseminated intravascular coagulation (5, 9, 30, 35).

With the advent of modern molecular identification techniques, the nomenclature within the genus *Trichosporon* has been substantially revised. While the major species previously encompassed *Trichosporon beigelii* (10, 32, 33), today, at least 13 *Trichosporon* species have been reported to be human pathogens. These include *T. asahii*, *T. asteroides*, *T. coremiiforme*, *T. cutaneum*, *T. dermatis*, *T. domesticum*, *T. faecale*, *T. inkin*, *T. japonicum*, *T. jirovecii*, *T. loubieri*, *T. montevideense*, and *T. mucoides* (5, 25). Yet the identification of *Trichosporon* and closely related yeasts to species level still largely relies on phenotype-based methods, which are insensitive and time-consuming (20). Further, none of the available commercial phenotypic identification systems include all new taxonomic categories or species in their databases. Specifically, the API 20C AUX (bioMérieux, Marcy l'Etoile, France) as well as the Vitek 2 Compact YST (bioMérieux) systems are able to identify only three *Trichosporon* species, *T. asahii*, *T. inkin*, and *T. mucoides*, which may lead to erroneous species assignment (6, 11, 17, 23, 24). Species identification is important for epidemiological purposes and to better define species-specific clinical associations (5, 11). In addition, certain *Trichosporon* species may be more resistant to antifungal drugs (2, 5, 25).

To enable accurate species identification, a number of molecular methods have been developed, of which DNA sequencing of the internal transcribed spacer (ITS) region, the D1/D2 domain of the 26S subunit of the rRNA gene region, and the intergenic spacer 1 (IGS1) region is the most frequently used (5, 7, 12, 19, 25, 26, 28–31). The IGS1 gene region has been particularly useful in phylogenetic studies and in delineating intraspecies variation. These data are fundamental to better understanding both species distribution and genotype differences that have been reported according to geographic region, such as that observed with *T. asahii* (5, 12, 19, 26, 28, 29).

In contrast to data from South and North America and Japan, studies examining the species distribution, phylogenetic diversity, and antifungal susceptibilities of *Trichosporon* pathogens in China are few. In the present study, we studied a collection of 45 clinical *Trichosporon* isolates obtained from

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TABLE 1. Identification, genotyping, and antifungal susceptibilities of 48 *Trichosporon* isolates

Strain ID[a]	Body site of isolation	Molecular ID (IGS1 genotype)[b,c]	ID by API 20C AUX/Vitek 2 Compact YST	MIC (μg/ml)				
				CAS	FLC	VRC	ITC	AMB
PUMCHBY15/CBS 2479	Unknown	*Trichosporon asahii* (genotype 1)	*T. asahii*	4	1	≤0.032	0.5	1
PUMCHBY28/ATCC 28592	Unknown	*Trichosporon cutaneum*	Unidentified	4	0.125	0.032	0.125	0.5
PUMCHMC31/CGMCC 2.1963	Unknown	*Trichosporon laibachii*	*Trichosporon mucoides*/unidentified	4	0.5	≤0.032	0.125	1
PUMCH6Z2374	Unknown[d]	*Trichosporon jirovecii* (genotype 1)	*Cryptococcus humicola*/*Cryptococcus laurentii*	16	8	0.064	0.5	0.5
PUMCH6Z10950	Sputum	*T. jirovecii* (genotype 2)	*C. humicola*/*C. laurentii*	16	1	0.032	0.25	2
PUMCHBY27	Blood	*Trichosporon japonicum* (genotype 1)	*T. asahii*	8	4	0.064	0.25	8
PUMCHBY11	Hand	*T. japonicum* (genotype 2)	Unidentified	16	1	0.032	0.25	4
PUMCHBY23	Blood	*Trichosporon inkin*	*T. inkin*/unidentified	8	1	0.032	0.125	0.5
PUMCHNJ32	Unknown[d]	*Trichosporon domesticum*	Unidentified	8	1	0.032	0.125	0.25
PUMCHBY20	Urine	*T. domesticum*	Unidentified/*T. asahii*	>16	4	0.125	0.5	0.5
PUMCHBY21	Sputum	*T. domesticum*	*T. inkin*/unidentified	>16	2	0.064	0.25	0.5
PUMCHBY22	Sputum	*T. domesticum*	*T. inkin*/unidentified	16	4	0.064	0.25	0.5
PUMCHBY24	Abscess	*Trichosporon dermatis*	*T. mucoides*/unidentified	16	32	0.5	0.5	1
PUMCH30404	Sputum	*T. asahii* (genotype 1)	*T. asahii*	8	8	0.064	1	1
PUMCH7Z7552	Liver drain	*T. asahii* (genotype 1)	*T. asahii*	8	2	0.125	1	1
PUMCH30407	Sputum	*T. asahii* (genotype 1)	*T. asahii*	8	2	0.064	1	1
PUMCHBY16	Urine	*T. asahii* (genotype 1)	*T. asahii*	8	4	≤0.032	1	1
PUMCHBY17	Sputum	*T. asahii* (genotype 1)	*T. asahii*	4	2	0.064	0.25	1
PUMCHBY18	Unknown[d]	*T. asahii* (genotype 1)	*T. asahii*/unidentified	4	1	≤0.032	0.25	1
PUMCHBY25	Skin (face)	*T. asahii* (genotype 1)	Unidentified	>16	32	0.25	2	0.25
PUMCHBY26	Tissue (face)	*T. asahii* (genotype 1)	Unidentified/*C. laurentii*	>16	16	0.064	0.25	0.064
PUMCHBY29	Liver	*T. asahii* (genotype 1)	*T. asahii*	16	16	0.5	1	≤0.032
PUMCH8W2360	Catheter tip	*T. asahii* (genotype 3)	*T. asahii*	8	4	0.064	1	1
PUMCH7R7615	Sputum	*T. asahii* (genotype 3)	*T. asahii*	8	1	0.064	1	1
PUMCH30406	Sputum	*T. asahii* (genotype 3)	*T. asahii*	8	1	0.5	1	1
PUMCH5Z6443	Lung	*T. asahii* (genotype 3)	*T. asahii*	8	4	0.5	1	1
PUMCH6Z8369	Unknown[d]	*T. asahii* (genotype 3)	*T. asahii*	4	1	0.125	1	1
PUMCH6W5203	Unknown[d]	*T. asahii* (genotype 3)	*T. asahii*	8	2	1	1	1
PUMCH8W2883	Urine	*T. asahii* (genotype 3)	*T. asahii*/*C. laurentii*	8	4	0.125	0.25	1
PUMCH5Z6527	Sputum	*T. asahii* (genotype 4)	*T. asahii*	16	2	0.064	0.5	1
PUMCH6Z6579	Sputum	*T. asahii* (genotype 4)	*T. asahii*	16	1	0.125	0.5	1
PUMCH6Z2782	Nasal secretions	*T. asahii* (genotype 4)	*T. asahii*	8	1	≤0.032	0.5	1
PUMCH6Z10766	Unknown[d]	*T. asahii* (genotype 4)	*T. asahii*	2	1	0.064	2	1
PUMCH6Z9690	Feces	*T. asahii* (genotype 4)	*T. asahii*	8	1	0.064	1	1
PUMCH7Z102	Sputum	*T. asahii* (genotype 4)	Unidentified/*T. asahii*	16	2	0.064	1	1
PUMCH30401	Sputum	*T. asahii* (genotype 4)	*T. asahii*	2	0.5	0.064	0.25	1
PUMCH30402	Sputum	*T. asahii* (genotype 4)	*T. asahii*	1	2	0.064	0.25	1
PUMCH30403	Sputum	*T. asahii* (genotype 4)	*T. asahii*/unidentified	4	1	0.064	1	1
PUMCH30405	Sputum	*T. asahii* (genotype 4)	*T. asahii*/unidentified	8	2	0.064	1	1
PUMCH30408	Sputum	*T. asahii* (genotype 4)	*T. asahii*/unidentified	8	2	0.064	0.5	1
PUMCH30409	Sputum	*T. asahii* (genotype 4)	*T. asahii*	8	2	0.064	1	1
PUMCH30410	Sputum	*T. asahii* (genotype 4)	*T. asahii*	8	2	0.064	1	1
PUMCHBY12	Sputum	*T. asahii* (genotype 4)	*T. asahii*/unidentified	>16	8	0.064	0.25	0.5
PUMCHBY13	Sputum	*T. asahii* (genotype 4)	*T. asahii*/unidentified	8	1	0.064	0.5	1
PUMCHBY14	Sputum	*T. asahii* (genotype 4)	*T. asahii*	2	1	≤0.032	0.25	1
PUMCHBY19	Abdominal fluid	*T. asahii* (genotype 4)	*T. asahii*	4	1	≤0.032	0.5	1
PUMCH8R5548	Sputum	*T. asahii* (genotype 4)	*T. asahii*	8	2	0.064	1	1
PUMCHJS30	Pleural fluid	*T. asahii* (genotype 6)	*T. asahii*	8	0.5	0.064	1	1

[a] ID, identification.
[b] By sequence analysis of the ITS region, D1/D2 domain, and IGS1 region (for detailed information, see Table S1 in the supplemental material).
[c] The genotypes of 36 *T. asahii* isolates are identified by IGS1 region sequencing (28, 29).
[d] These isolates were from clinical specimens, but sites of isolation were not available.

three hospitals in China along with three well-characterized reference strains. Species identification of all isolates was undertaken by sequence analysis of the ITS region, D1/D2 domain, and IGS1 region and compared with that obtained by the API 20C AUX and Vitek 2 Compact YST methods. Testing of the antifungal susceptibilities of *Trichosporon* isolates to five antifungal agents was also performed.

MATERIALS AND METHODS

***Trichosporon* strains and DNA extraction.** Forty-eight *Trichosporon* isolates were studied, including (i) 3 reference strains, namely, *T. asahii* CBS 2479 (PUMCH strain identification no. PUMCHBY15), *T. cutaneum* ATCC 28592 (PUMCH strain identification no. PUMCHBY28), and *T. laibachii* CGMCC 2.1963 (PUMCH strain identification no. PUMCHMC31) (Table 1), and (ii) 45 clinical isolates collected from three hospitals (Peking Union Medical College Hospital, Peking University First Hospital, and First Affiliated Hospital of Chinese People's Liberation Army General Hospital) in Beijing, China. Of the 45 clinical isolates, 22 strains were isolated from sputum, 12 from tissue or body fluid, 3 from urine, 2 from blood, and 6 from unknown clinical sources (Table 1). All isolates were initially identified by the API 20C AUX and Vitek 2 Compact YST systems according to the manufacturers' instructions (Table 1) and then subjected to DNA sequencing (see below). DNA was extracted from pure cultures of each of the isolates as described previously (16) and stored at −20°C before use.

PCR and DNA sequencing. Amplification of the ITS region, D1/D2 domain, and IGS1 region was performed as previously described with primer pairs ITS1/ITS4, F63/R635, and 26SF/5SR, respectively (7, 29). A total of 8 μl of amplified PCR products was visualized on a 2% agarose gel after staining with SYBR safe

TABLE 2. Performance of the commercial biochemical methods in the present study[a]

Species by molecular ID	Total	No. (%) of isolates					
		API 20C AUX			Vitek 2 Compact YST		
		C	U	M	C	U	M
T. asahii[b]	36	33 (91.7)	3 (8.3)	0	27 (75.0)	7 (19.4)	2 (5.6)
T. inkin[b]	1	1 (100)	0	0	0	1 (100)	0
T. laibachii[c]	1	0	0	1 (100)	0	1 (100)	0
T. cutaneum[c]	1	0	1 (100)	0	0	1 (100)	0
T. dermatis[c]	1	0	0	1 (100)	0	1 (100)	0
T. domesticum[c]	4	0	2 (50)	2 (50)	0	3 (75)	1 (25)
T. japonicum[c]	2	0	1 (50)	1 (50)	0	1 (50)	1 (50)
T. jirovecii[c]	2	0	0	2 (100)	0	0	2 (100)
Total species claimed by databases	37	34 (91.9)	3 (8.1)	0	27 (73.0)	8 (21.6)	2 (5.4)
Total species unclaimed by databases	11	0	4 (36.4)	7 (63.6)	0	7 (63.6)	4 (36.4)
Total	48	34 (70.8)	7 (14.6)	7 (14.6)	27 (56.3)	15 (31.2)	6 (12.5)

[a] Abbreviations: ID, identification; C, isolates that were correctly identified; U, isolates that were unidentified; M, isolates that were misidentified.
[b] Species claimed by databases of API 20C AUX and Vitek 2 Compact YST systems.
[c] Species not claimed by databases of API 20C AUX and Vitek 2 Compact YST systems.

DNA gel stain (Invitrogen, Mt. Waverley, VIC, Australia). The PCR products were then sequenced by Tsingke Co. Ltd. (Beijing, People's Republic of China) with the DNA analyzer ABI 3730XL system (Applied Biosystems, Foster City, CA) in both directions using the PCR amplification primers.

Sequencing-based species identification and phylogenetic analysis. All DNA sequence chromatograms were checked manually to ensure high-quality sequences. For species identification, sequences of the ITS region, D1/D2 domain, and IGS1 region obtained in this study were queried against sequences of type strains for all *Trichosporon* species in the Centraalbureau voor Schimmelcultures (CBS) Fungal Biodiversity Center database using pairwise sequence alignment with the BioloMICSNet software (http://www.cbs.knaw.nl/collections/BioloMICSSequences.aspx). The sequence of each target gene region and concatenated sequences of the ITS region and D1/D2 domain were then aligned using Clustal W software (36) and adjusted manually to form the consensus sequences for all 48 isolates. Phylogenetic trees were computed with MEGA, version 4 (Molecular Evolutionary Genetic Analysis software, version 4.0.2; http://www.megasoftware.net) using maximum-parsimony analysis (34), with all positions containing gaps and missing data eliminated from the data set. ITS region, D1/D2 domain, and IGS1 region sequences from the whole-genome sequence of *Cryptococcus neoformans* var. *neoformans* strain B-3501A (15) were used as outgroups to generate the maximum-parsimony trees (MPTs). The IGS1 region was further targeted to determine the genotypes of the *T. asahii* isolates as described previously (26, 28, 29).

Antifungal susceptibility testing. Antifungal susceptibility testing was performed according to the Clinical and Laboratory Standards Institute (CLSI; formerly NCCLS) document M27-A3 and M27-S3 broth microdilution method (21, 22). The following antifungal drugs were tested at the indicated concentration ranges: amphotericin B (AMB; North China Pharmaceutical Group Corporation, Shijiazhuang, China), 0.032 to 16 μg/ml; caspofungin (CAS; Merck Research Laboratories, Rahway, NJ), 0.032 to 16 μg/ml; fluconazole (FLC; Pfizer Incorporated, New York, NY), 0.125 to 64 μg/ml; itraconazole (ITC; National Institute for Food and Drug Control, Beijing, China), 0.032 to 16 μg/ml; and voriconazole (VRC; Pfizer Incorporated), 0.032 to 16 μg/ml. The microdilution plates were incubated at 35°C, except for those for *T. cutaneum* ATCC 28592 (strain PUMCHBY28), where plates were incubated at 28°C as described previously (14). All plates were observed for visible growth at 48 h. Microdilution wells were scored with the aid of a reading mirror, and the growth in each well compared was with that of the growth-control (drug-free) well (21). *Candida parapsilosis* ATCC 22019 and *Candida krusei* ATCC 6258 were used as the quality control strains.

Nucleotide sequence accession numbers. New data for sequences within the ITS region, D1/D2 domain, and IGS1 region generated in this study have been deposited in the GenBank database with accession numbers EU863532 to EU863579 (for ITS region), EU882088 to EU882103 (for D1/D2 domain), and JF302977 to JF303024 (for IGS1 region).

RESULTS

Sequencing-based identification of *Trichosporon* species. All 48 *Trichosporon* isolates were identified by querying their unknown ITS, D1/D2, and IGS1 sequences against sequences of *Trichosporon* type strains in the CBS Fungal Biodiversity Center database, and results are summarized in Table S1 in the supplemental material.

The three loci correctly identified the reference strains to species level (Table 1; see Table S1 in the supplemental material). Among 45 clinical isolates, six species were identified. *Trichosporon asahii* was the most common species (35 isolates, 77.8%), followed by *T. domesticum* (4 isolates, 8.9%), *T. japonicum* and *T. jirovecii* (2 isolates each, 4.4%), and *T. dermatis* and *T. inkin* (1 isolate each, 2.2%) (Table 1; see Table S1 in the supplemental material). Sequencing of both the ITS and IGS1 regions successfully identified all 45 clinical isolates. D1/D2 domain sequences correctly assigned 44 of 45 (97.8%) strains to species level, with the exception of strain PUMCHBY24, which was identified as *T. mucoides* but as *T. dermatis* by ITS and IGS1 sequencing; *T. dermatis* was assigned the correct species for this strain (see Table S1 in the supplemental material).

Comparison of phenotype-based and molecular identification. Identification results for 48 *Trichosporon* isolates tested with the Vitek 2 Compact YST and API 20C AUX systems are summarized in Tables 1 and 2. As the databases of both the API 20C AUX and Vitek 2 Compact YST systems contain only three *Trichosporon* species (*T. asahii*, *T. inkin*, and *T. mucoides*), isolates in the present study were further subgrouped as "species claimed by databases" (37 isolates, two species) and "species unclaimed by databases" (11 isolates, six species) for analysis (Table 2).

Compared with the results obtained by our three-locus sequencing scheme, the API 20C AUX system correctly identified 34 of 37 (91.9%) isolates among species claimed by databases, with the remaining three isolates unidentified (with their biochemical profiles yielding either "low discrimination" or "no identification") and no isolates misidentified, while among

TABLE 3. Tree statistics for the three genes studied[a]

Genetic locus	Length (bp)	No. of PICs	No. of PICs/bp	No. of MPTs	MPT length (steps)	CI	RI	No. of sequence types/ genotypes identified
ITS	595	50	0.08	981	232	0.71	0.93	10
D1/D2	663	54	0.08	605	153	0.82	0.93	8
ITS + D1/D2	1,258	104	0.08	300	203	0.83	0.92	10
IGS1	1,110	182	0.16	213	110	0.77	0.84	13

[a] Abbreviations: CI, consistency index; RI, retention index.

species unclaimed by databases, 4 of 11 isolates were correctly shown to be unidentified but 7 were misidentified (Tables 1 and 2). The ability of the Vitek 2 Compact YST system to identify *Trichosporon* spp. among species claimed by databases was even lower: only 27 *T. asahii* isolates (75.0% of 36 *T. asahii* isolates studied) were correctly identified. However, among species unclaimed by databases, 7 of 11 isolates were correctly shown to be unidentified but 4 isolates were misidentified (Tables 1 and 2).

Phylogenetic analysis of IGS1 region and genotyping. Phylogenetic analysis was performed on the basis of the results of sequence analysis of the ITS region (595 bp), D1/D2 domain (663 bp), and IGS1 region (1,110 bp) as well as by using concatenated sequences of the ITS region and D1/D2 domain (Table 3). On the basis of the number of parsimony informative characters (PICs) per bp (Table 3), the IGS1 region was more phylogenetically informative than either the ITS region or D1/D2 domain. MPTs were generated for the IGS 1 region (Fig. 1, left MPT) and ITS region plus D1/D2 domain (Fig. 1, right MPT) rooted by the outgroup *C. neoformans* var. *neoformans* strain B-3501A.

Among the 48 isolates, the IGS1 region recognized 13 sequence types/genotypes (Table 3). Genotypes of *T. asahii* isolates were assigned as described by Sugita et al. (28) and Rodriguez-Tudela et al. (26). *Trichosporon asahii* reference strain PUMCHBY15/CBS 2479 was correctly classified as genotype 1 (5). The remaining 35 clinical *T. asahii* isolates belonged to four genotypes, namely, genotype 1 (9 isolates, 25.7%), genotype 3 (7 isolates, 20.0%), genotype 4 (18 isolates, 51.4%), and genotype 6 (1 isolate, 2.9%) (Table 1 and Fig. 1). Four *T. domesticum* isolates studied had a single IGS1 sequence type, while two sequence types were found for both *T. japonicum* strains and two *T. jirovecii* strains (Table 1; see Table S1 in the supplemental material).

Antifungal susceptibility testing. The susceptibilities of 48 isolates representing eight species (on the basis of the molecular identification result) to five antifungal agents are shown in Table 1. MIC ranges (and geometric mean [GM] MICs) were 0.125 to 32 μg/ml (1.09 μg/ml) for AMB, 1 to 16 μg/ml (9.15 μg/ml) for CAS, 0.125 to 8 μg/ml (3.97 μg/ml) for FLC, 0.125 to 2 μg/ml (0.66 μg/ml) for ITC, and 0.032 to 1 μg/ml (0.12 μg/ml) for VRC (Table 1). CAS MICs for all *Trichosporon* isolates tested were consistently high (≥2 μg/ml for all strains tested except one *T. asahii* isolate, for which the CAS MIC was 1 μg/ml). Two *T. japonicum* isolates exhibited AMB MICs of ≥4 μg/ml, while all *T. asahii* isolates had AMB MICs of ≤1 μg/ml (GM MIC, 0.91 μg/ml). With regard to the azoles, FLC MICs for 27 *Trichosporon* isolates were ≥2 μg/ml. GM MICs of FLC were 3.97 μg/ml for all tested isolates and 3.67 μg/ml for the most common species, *T. asahii.* ITC and VRC were the most potent agents *in vitro* against all *Trichosporon* spp., particularly VRC, with GM MICs of 0.12 μg/ml.

DISCUSSION

Trichosporon species are medically important yeast pathogens, but their prevalence and species distribution have not been well studied in China. A recent study of approximately 800 isolates causing invasive yeast infections in China has estimated that 1% of all such infections are due to *Trichosporo*n spp. (China Hospital Invasive Fungal Surveillance Net [CHIF-NET], 2009-2010, unpublished data). In the present study, we note that commercial biochemical-based methods may be limited in their capacity to identify *Trichosporon* species, even those that are claimed to be included within their databases. We confirmed that both the ITS and IGS1 sequences are suitable targets for accurate species identification by sequencing (with 100% sensitivity) (29, 30; this study). Further, the IGS1 locus also provided discriminatory information with regard to strain variation within the predominant species, *T. asahii*, encountered in China.

To date, the identification of *Trichosporon* yeasts in the clinical mycology laboratory has largely relied on morphological, physiological, and biochemical characteristics (20). Specifically, API test strips have been long considered the "gold standard" for clinical diagnosis (3, 20) in many clinical microbiology laboratories in China. However, neither system can assign *Trichosporon* isolates to species level among species unclaimed by databases (misidentification of 63.6% by API 20C AUX system and 36.4% by Vitek 2 Compact YST system, respectively; Table 2). The present study also showed that among *Trichosporon* species claimed by the databases of commercial systems, not all isolates were correctly identified (no identification for 8.1% and 21.6% with API 20C AUX and Vitek 2 Compact YST, respectively, and 5.4% misidentification for Vitek 2 Compact YST; Table 2).

Our results confirmed the findings of others (5, 11, 25, 29) that molecular methods have good potential to provide reliable, accurate species identification. Earlier studies exploited sequence variation within the ITS region and D1/D2 domain between species for species assignment (7, 25, 30, 31). However, as *Trichosporon* species are phylogenetically very closely related to each other, several reports indicated that sequence analyses of the ITS and D1/D2 regions were unable to unambiguously distinguish between all species (5, 11, 28, 29). In the present study, the ITS region identified all 48 isolates studied,

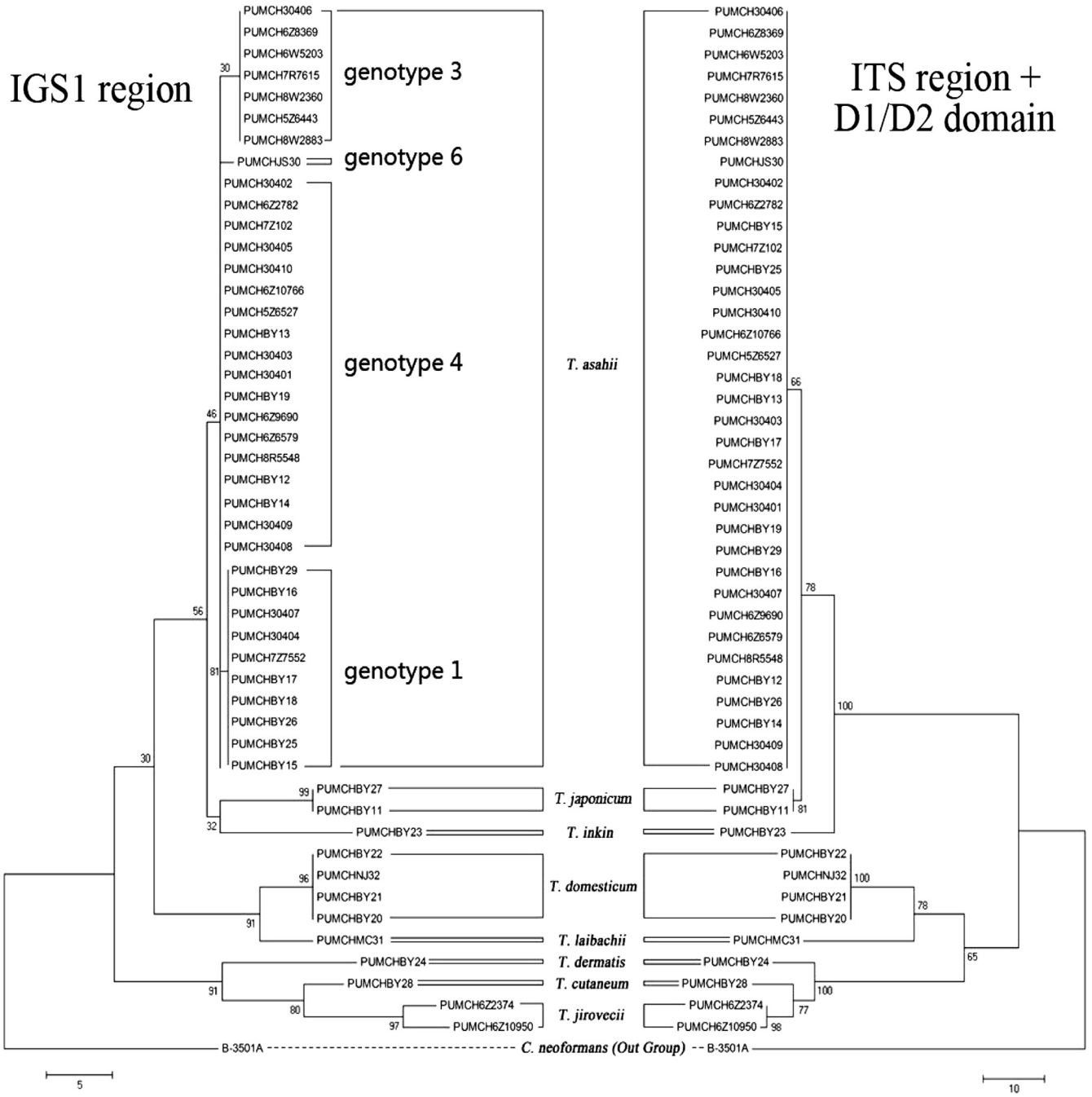

FIG. 1. MPTs by comparison of sequences of the IGS1 region and the ITS region plus D1/D2 domain of the 26S rRNA gene. ITS region, D1/D2 domain, and IGS1 region sequences from the whole-genome sequence of *Cryptococcus neoformans* var. *neoformans* strain B-3501A were used as outgroups. Eight *Trichosporon* species identified in the present study are indicated; and four *T. asahii* IGS1 genotypes are marked in the IGS1 MPT (left tree) as well. Scale bars indicate branch lengths corresponding to 5 nucleotide changes for the IGS1 region (bottom left) and 10 nucleotide changes for the ITS region plus D1/D2 domain (bottom right).

and the D1/D2 domain identified 47 of 48, though for certain *Trichosporon* species, distinction by ITS region and D1/D2 domain was based on differences of only a few nucleotide base pairs or even a single nucleotide base pair. For example, the ITS sequences of two *T. japonicum* isolates in the present study were 100% (541/541) identical to the ITS sequence of *T. japonicum* CBS 8641^T (see Table S1 in the supplemental material), with a 1-bp difference compared to the ITS sequence of *T. asteroides* CBS 2481^T (540/541, 99.8%) and only a 3-bp difference compared to the ITS sequence of *T. asahii* CBS 2479^T (538/541, 99.4%). Thus, among closely related *Trichosporon* spp., such as *T. japonicum* and *T. asteroides*, if a cutoff value of less than 100% was employed by sequencing of the ITS region and D1/D2 domain, there could be a risk of mis-

identification, and sequencing of an additional locus, e.g., the IGS1 region, should be considered. The IGS1 region was characterized by a greater degree of nucleotide polymorphism than either the ITS region or D1/D2 domain (5, 26, 28, 29); the present study showed that IGS1 sequences with sequence identities as low as 97.1% could still belong to the same species (as demonstrated for *T. asahii* genotypes 1, 3, and 6; see Table S1 in the supplemental material) and IGS1 sequences with sequence identities even as low as 90.9% could still belong to the same species (as demonstrated for *T. jirovecii* genotype 2 and *T. jirovecii* CBS 6864^T; see Table S1 in the supplemental material). Thus, when using the IGS1 region for species identification, a lower cutoff value should be employed.

Of interest, one of the two *T. japonicum* isolates studied (PUMCHBY27) was identified as *T. asahii* by commercial identification systems (Table 1). In the first reported isolation of *T. japonicum* from a clinical specimen, the isolate was likewise misidentified as *T. asahii* (1). We were also unable to discriminate between *T. dermatis* and *T. mucoides* by D1/D2 sequencing (Table 1; see Table S1 in the supplemental material). This is in keeping with the observations of others reporting on the potential misidentification of *T. dermatis* as *T. mucoides* by ITS sequencing (5, 11). Since these two species have different propensities to cause superficial versus invasive infections (5, 11), accurate species identification is important for clinical diagnosis and in fungal surveillance.

A major finding of the present study is that, in contrast to the other two regions analyzed, examination of sequence variation within the IGS1 region enabled the accurate identification of all 48 study isolates, consistent with previous findings (5, 11, 12, 19, 26, 28, 29). Indeed, eight species were identified among the 48 test strains, including six species among 45 clinical isolates, supporting previous reports that the sequence polymorphisms within the IGS1 region provide powerful and discriminatory information for distinguishing between phylogenetically closely related species and that the IGS1 region is the preferred target for sequence-based identification of *Trichosporon* species (1, 5, 25, 28, 29).

Further, IGS1 sequence analysis also shows great potential as an epidemiological tool. For instance, the geographic distribution of different genotypes of *T. asahii* isolates by IGS1 sequencing has been described by a number of studies (5, 12, 19, 26, 28, 29). In the present study, among 35 *T. asahii* Chinese isolates, the most predominant genotype was genotype 4 (51.4%), previously only rarely found (approximately 1.0 to 4.5%) in Japan, South America, and Turkey (5, 12, 29). Other genotypes observed in this study were genotype 1 (25.7%), genotype 3 (20.0%), and genotype 6 (2.9%). Of note, IGS1 genotype 1 is reported to be the most predominant in Japan as well as in South America and Europe (57% to 87%) (5, 12, 26, 28, 29), and genotype 3 is reported to be common in the United States (about 60%) (29).

Consistent with the findings of others (5, 12, 19), MICs to CAS for all isolates were high (GM MIC, 9.15 μg/ml; Table 1). This *in vitro* finding is consistent with the increasing number of reports of disseminated *Trichosporon* infection in patients receiving echinocandin antifungal treatment (8). In contrast, VRC (GM MIC, 0.12 μg/ml) and ITC (GM MIC, 0.66 μg/ml) were the most active anti-*Trichosporon* drugs (Table 1) (5, 12, 19, 25). With the exception of two strains of *T. japonicum* and one strain of *T. jirovecii* that had AMB MICs of ≥2 μg/ml, all the other clinical isolates (including all *T. asahii* isolates studied) had low MICs to AMB (Table 1); these findings are in contrast to the low susceptibility to AMB for *T. asahii* isolates reported by others (2, 5, 12, 25). FLC was less active than VRC and ITC (MIC range, 0.125 to 8 μg/ml; GM MIC, 3.97 μg/ml), with the GM MIC being higher than that reported in a Brazilian study (1.1 μg/ml), although it was lower than that in a study from Turkey (12.5 μg/ml) (5, 12). This suggests that where possible, antifungal susceptibility testing should be performed on all clinical isolates not only to guide therapy but also to document local epidemiological trends.

Limitations of the present study include the small number of isolates available for study and the fact that all clinical isolates were collected from hospitals in Beijing. Further collection and study of *Trichosporon* isolates in China are ongoing on the basis of the nationwide surveillance program CHIF-NET, which seeks to describe the clinical and molecular epidemiology of a range of yeast pathogens.

In conclusion, the present study provided accurate species identification of a Chinese collection of *Trichosporon* isolates by combining the use of ITS, D1/D2, and IGS1 sequencing. Eight species were identified, including six species among clinical isolates. Phenotypic methods for *Trichosporon* species identification, including API 20C AUX and Vitek 2 Compact YST, were not as accurate as molecular methods. Phylogenetic analysis and genotyping by IGS1 sequencing showed a significant subgeographic difference among *Trichosporon* clinical isolates in China, with genotype 4 being the predominant genotype among *T. asahii* isolates. VRC was the most active drug *in vitro* against all *Trichosporon* species, while CAS demonstrated poor activity.

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