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Genetic Diversity and Biochemical Characteristics of *Trichosporon asahii* Isolated from Clinical Specimens, Houses of Patients with Summer-Type-Hypersensitivity Pneumonitis, and Environmental Materials

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***Trichosporon asahii*, which is distributed in the environment, is the major causative agent of the opportunistic infection trichosporonosis, and it also causes summer-type hypersensitivity pneumonitis (SHP). Random amplification of polymorphic DNA analysis was used to determine the intraspecies diversity of 39 *T. asahii* isolates from clinical specimens, SHP patients' houses, and environmental materials. The three primers used revealed 46 polymorphic bands. A phenogram was generated by the unweighted pair-group method with arithmetic mean. Clinical isolates formed a cluster, characterized by a 90% matching coefficient, but they did not cluster with strains isolated from SHP patients' houses or environmental sources. In addition, the biochemical characteristics of 86 strains from three sources were examined with 31 compounds using an ID32C kit, and a phenogram was constructed. The phenogram consisted of three major clusters. Cluster I included most of the clinical SHP isolates, and cluster II included most of the environmental isolates. Cluster III contained only one strain. A remarkable difference was found in the abilities of the strains belonging to clusters I and II to utilize six compounds. These results suggest that the genetic diversity and biochemical characteristics of *T. asahii* seem to be related to the source of the isolate. We also found a specific DNA fragment for the clinical isolates and strains isolated from SHP patients' houses.**

The basidiomycetous anamorphic yeast *Trichosporon asahii* Akagi ex Sugita et al. is the major causative agent of fungemia due to *Trichosporon* species in immunocompromised patients (7, 8). This infection is associated with a high mortality rate and a poor prognosis (20). Neutropenia due to cytotoxic chemotherapy is the most common risk factor for deep-seated trichosporonosis, and neutropenic patients are more likely to have fungemia or disseminated infection than nonneutropenic patients. *T. asahii* also causes summer-type hypersensitivity pneumonitis (SHP) (1, 2). SHP follows the development of type III or type IV allergies by repeated inhalation of *Trichosporon* arthroconidia, which often contaminate home environments during the summer months. In western and southern Japan, the summer is hot, humid, and rainy. Such conditions favor the growth of *Trichosporon* species, and most patients initially experience symptoms during the summer. Although SHP is considered peculiar to Japan, a case was recently reported in a neighboring country (22). *Trichosporon* is widely distributed throughout the environment, especially in soil. We have previously reported that *T. asahii* is a common environmental pathogen (19).

Many investigations of the intraspecies diversity and epidemiology of *Candida albicans* and *Cryptococcus neoformans* using random amplified polymorphic DNA (RAPD) analysis, hybridization with specific probes such as Ca3, and multilocus enzyme electrophoresis (MLEE) have been reported (3–5, 12–15, 21). They include studies examining the origins of nosocomial infection (12), a generic comparison between bloodstream and nonbloodstream isolates (5), a study of the hospital specificity or regional specificity of the isolates (14), and comparison of the genotypes and fluconazole susceptibilities of the isolates (13, 21). In contrast, there are only a few reports for *Trichosporon* species. Isoenzyme profiles, restriction fragment length polymorphisms (RFLP) of ribosomal DNA (rDNA), and analysis of glucuronoxylomannan polysaccharide antigen revealed that blood, superficial, and environmental isolates of *Trichosporon beigelii* (synonymous with *Trichosporon cutaneum*) were distinct from each other (9, 10). However, *T. beigelii* is a taxonomically highly heterogeneous species; at present, this species has been reclassified into more than 10 species (6).

In this study, we examined the genetic diversity and biochemical characteristics of *T. asahii* strains isolated from various sources, including clinical specimens, SHP patients' houses, and environmental materials.

MATERIALS AND METHODS

***T. asahii* isolates.** Of the 86 isolates identified as *T. asahii*, 42 were derived from clinical specimens (ascites, blood, feces, lung, pleural fluid, skin, and urine). They were obtained from eight centers in Japan and the United States. Seventeen were from the homes of 17 SHP patients, and 27 were from soil (Table 1). They were identified by PCR with *T. asahii*-specific primers or by direct sequence analysis of internal transcribed spacer (ITS) regions of the rRNA gene (17, 18)

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TABLE 1. *T. asahii* isolates and their sources, API profiles, and fingerprinting genotypes

Strain	Site	Source[a]	API profile	Fingerprinting genotype[b] with primer:		
				M13	OPE1	RC8
Clinical isolates						
C.I.1	Urine	JU	7 7 5 7 6 7 5 3 3 7	0 0 0 0 1 0 0 0 0 0 0 0 1 0 1 0 0 0 1 0 1 0	0 0 0 0 1 1 0 0 1 0	1 0 0 0 0 0 0 0 1 0 1 0 0 0
C.I.2	Urine	JU	7 7 5 7 6 6 5 2 2 7	0 0 0 0 1 0 0 0 0 0 0 0 1 0 1 0 0 0 1 0 1 0	0 0 0 0 0 0 0 0 0 1 0	1 0 0 0 1 0 0 0 1 0 1 0 1 0
C.I.3	Blood	TMBH	7 7 5 7 6 7 1 3 2 7	0 1 0 0 1 0 0 0 0 0 0 0 1 0 1 0 0 0 1 0 1 0	0 0 0 1 1 1 0 0 1 0	1 1 0 0 1 0 0 0 1 0 1 0 1 0
C.I.4	Sputum	NU	7 7 5 7 6 4 5 2 2 7	0 0 0 0 1 0 0 0 0 0 0 0 1 0 1 0 0 0 1 0 1 0	0 0 0 1 1 1 0 0 1 0	1 0 0 0 0 0 0 0 1 0 1 0 1 0
C.I.5	Pleural fluid	NU	5 7 5 7 6 7 5 3 2 5	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 1 0	0 0 0 0 0 0 0 0 1 0	0 0 0 0 0 0 0 0 1 0 1 0 1 0
C.I.6	Urine	NU	7 7 5 7 6 6 5 2 2 5	0 0 0 0 1 0 0 0 0 0 0 0 1 0 1 0 0 0 1 0 1 0	0 0 0 0 1 1 0 0 1 0	0 0 0 0 0 0 0 0 1 0 1 0 1 0
C.I.7	Sputum	OMU	7 7 5 7 6 4 5 2 2 7	0 0 0 0 1 0 0 0 0 0 0 0 1 0 1 0 0 0 1 0 1 0	0 0 0 0 1 1 0 0 1 0	1 0 0 0 0 0 0 0 1 0 1 0 1 0
C.I.8	Urine	OMU	7 7 5 7 6 4 5 2 2 7	0 0 0 0 0 0 0 0 0 0 0 0 1 0 1 0 0 0 1 0 1 0	0 0 0 0 0 1 0 0 1 0	0 0 0 0 0 0 0 0 1 0 1 0 1 0
C.I.9	Ascites	OMU	7 7 5 7 6 4 5 3 2 5	0 0 0 0 1 0 0 0 0 0 0 0 1 0 1 0 0 0 1 0 1 0	0 0 0 0 1 1 0 1 1 0	0 0 0 0 1 0 0 0 1 0 1 0 1 0
C.I.10	Feces	OMU	7 7 5 7 6 4 5 2 2 7	0 0 0 0 0 0 0 0 0 0 0 0 1 0 1 0 0 0 1 0 1 0	0 0 0 0 0 0 0 0 0 1 0	0 0 0 0 0 0 0 0 1 0 1 0 1 0
C.I.11	Lung	KU	7 7 5 7 6 4 5 2 2 5	0 0 0 0 1 0 0 0 0 0 0 0 1 0 1 0 0 0 1 0 1 0	0 0 0 1 1 1 0 0 1 0	1 0 0 0 1 0 0 0 1 0 1 0 1 0
C.I.12	Urine	KULM	7 7 5 7 6 4 5 2 2 7	0 0 0 0 1 0 0 0 0 0 0 0 1 0 1 0 0 0 1 0 1 0	0 0 0 1 1 1 0 0 1 0	1 0 0 0 1 0 0 0 1 0 1 0 1 0
C.I.13	Ascites	JU	7 7 5 7 6 4 5 2 2 7	0 0 0 0 0 0 0 0 0 0 0 0 1 0 1 0 0 0 1 0 1 0	1 0 0 1 1 1 0 0 1 0	1 0 0 0 1 0 0 0 1 0 1 0 1 0
C.I.14	Feces	NYSDH 297-87[c]	7 7 5 7 6 4 5 3 2 7	0 0 0 0 1 0 0 0 0 0 0 0 1 0 1 0 0 0 1 0 1 0	0 0 0 1 1 1 0 1 1 0	1 0 0 1 1 0 0 0 1 0 1 0 1 0
C.I.15	Blood	UMSMT2[c]	7 7 5 7 7 6 5 2 3 5	0 0 0 0 1 0 0 0 0 0 0 0 1 0 1 0 0 0 1 0 1 0	0 0 0 1 1 1 0 0 1 0	1 0 0 0 0 0 0 0 1 0 1 0 1 0
C.I.16	Blood	JU	7 7 5 7 6 5 3 3 7	0 0 0 0 1 0 0 0 0 0 0 0 1 0 1 0 0 0 1 0 1 0	0 0 0 0 0 1 0 0 1 0	0 0 0 0 0 0 0 0 1 0 1 0 1 0
C.I.17	Blood	JU	7 7 5 7 6 4 5 2 2 5	0 0 0 0 0 0 0 0 0 0 0 0 1 0 1 0 0 0 1 0 1 0	0 0 0 0 0 0 0 0 0 1 0	0 0 0 0 0 0 0 0 1 0 1 0 0 0
C.I.18	Blood	JU	7 7 5 7 6 6 5 2 2 7	0 0 0 0 1 0 0 0 0 0 0 0 1 0 1 0 0 0 1 0 1 0	0 0 0 0 0 1 0 0 1 0	0 0 0 0 0 0 0 0 1 0 1 0 0 0
C.I.19	Urine	OMU	7 7 5 7 6 4 5 2 2 5			
C.I.20	Feces	OMU	7 7 5 6 6 4 5 3 2 5			
C.I.21	Blood	OMU	5 7 5 7 6 5 5 3 2 5			
C.I.22	Urine	OMU	7 7 5 7 6 4 5 2 2 5			
C.I.23	Urine	OMU	7 7 5 7 6 4 5 2 2 5			
C.I.24	Urine	NU	7 7 5 7 6 6 5 2 2 5			
C.I.25	Skin	NU	7 7 5 7 6 7 5 3 2 5			
C.I.26	Feces	NU	7 7 5 7 7 7 5 2 3 5			
C.I.27	Skin	NU	7 7 5 7 6 7 5 2 2 5			
C.I.28	Blood	NU	3 7 1 3 6 0 5 2 2 7			
C.I.29	Blood	NU	7 7 5 7 2 4 5 2 2 5			
C.I.30	Blood	NU	7 7 5 7 6 6 5 2 2 5			
C.I.31	Pleural fluid	NU	7 7 5 7 6 6 5 3 2 5			
C.I.32	Urine	NU	7 7 5 7 6 4 1 3 2 5			
C.I.33	Urine	NU	7 7 1 7 6 4 5 2 2 5			
C.I.34	Urine	NU	5 7 5 7 6 4 5 2 2 5			
C.I.35	Urine	NU	5 7 5 7 6 5 5 3 2 5			
C.I.36	Urine	NU	7 7 5 7 6 7 5 3 2 5			
C.I.37	Urine	NU	7 7 5 7 6 6 5 2 2 5			
C.I.38	Blood	JU	7 7 5 7 6 4 5 2 2 7			
C.I.39	Blood	JU	7 7 5 7 6 6 5 3 2 5			
C.I.40	Sputum	NYSDH958-85[c]	7 7 5 7 7 6 5 3 2 5			
C.I.41	Blood	UMSMT3[c]	7 7 5 7 7 6 5 2 3 5			
C.I.42	Urine	KULM	7 7 5 7 6 4 5 2 2 7			
Strains isolated from SHP patients' houses						
SHP 1	SHP patient's house	Tokyo, Japan	7 7 5 7 7 6 5 3 2 5	0 0 0 0 0 0 0 0 1 0 0 0 0 1 0 0 1 0 0 0 0 0	0 0 0 0 0 0 0 0 1 1	0 0 0 0 0 0 1 0 0 1 0 0 0 0
SHP 2	SHP patient's house	Kumamoto, Japan	7 7 5 7 6 4 5 3 2 5	1 0 0 0 1 0 0 0 0 0 0 0 1 0 1 0 0 0 1 0 1 0	0 0 0 0 1 1 0 1 0 0	0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 1
SHP 3	SHP patient's house	Kumamoto, Japan	7 7 5 7 6 6 5 3 2 5	0 1 0 0 1 0 0 0 0 0 0 0 1 0 1 0 0 0 1 0 1 0	0 0 0 0 1 0 0 1 0 0	0 0 0 0 0 0 0 0 0 0 0 0 1 0 1
SHP 4	SHP patient's house	Kumamoto, Japan	7 7 5 7 6 6 5 3 2 5	0 0 0 1 0 0 1 1 0 0 1 0 0 0 1 0 0 1 1 1 0 0	0 0 0 1 1 0 1 0 0 0	0 0 0 1 0 1 0 0 1 0 0 1 0 1
SHP 5	SHP patient's house	Kumamoto, Japan	7 7 5 7 6 4 5 3 2 5	0 0 0 0 0 0 0 0 0 0 0 0 1 0 1 0 0 0 1 0 0 0	0 0 0 0 0 0 0 0 1 0 0	0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 1
SHP 6	SHP patient's house	Kumamoto, Japan	7 7 5 7 6 4 5 3 3 7	0 0 0 0 1 0 0 0 0 0 0 0 1 0 1 0 0 0 1 0 1 0	0 0 0 0 1 1 1 0 0 1 0	0 0 1 0 0 1 0 0 1 0 1 0 0 0
SHP 7	SHP patient's house	Kumamoto, Japan	7 7 5 7 7 4 5 3 3 5	0 0 0 0 1 0 0 0 0 0 0 0 1 0 1 0 0 0 1 0 1 0	0 0 0 0 1 1 0 0 1 0	0 0 0 0 0 1 0 0 1 0 1 0 0 0
SHP 8	SHP patient's house	Kumamoto, Japan	7 7 5 7 7 4 5 3 3 5	0 1 0 0 1 0 0 0 0 0 0 0 1 0 1 0 0 0 1 0 1 0	0 0 0 1 1 1 0 0 1 0	0 0 0 0 1 0 0 0 1 0 1 0 0 0
SHP 9	SHP patient's house	Kumamoto, Japan	7 7 5 7 6 6 5 2 2 7	0 0 0 0 1 0 0 0 0 0 0 0 1 0 1 0 0 0 1 0 1 0	0 0 0 0 1 1 1 1 0 0	0 0 0 0 1 0 0 0 1 0 1 0 0 0

SHP 10	SHP patient's house	Kumamoto, Japan	7 7 5 7 7 6 5 3 2 5			
SHP 11	SHP patient's house	Kumamoto, Japan	7 7 5 7 6 4 5 3 2 5			
SHP 12	SHP patient's house	Kumamoto, Japan	7 7 5 7 7 4 5 3 3 5			
SHP 13	SHP patient's house	Kumamoto, Japan	7 7 5 7 6 6 5 3 2 5			
SHP 14	SHP patient's house	Kumamoto, Japan	7 7 5 7 6 6 5 3 2 5			
SHP 15	SHP patient's house	Kumamoto, Japan	7 7 5 7 6 4 5 3 3 7			
SHP 16	SHP patient's house	Kumamoto, Japan	7 7 5 7 6 4 5 3 2 5			
SHP 17	SHP patient's house	Kumamoto, Japan	7 7 5 7 7 4 5 3 3 5			
Environmental						
SHP 10	SHP patient's house	Kumamoto, Japan	7 7 5 7 7 6 5 3 2 5			
SHP 11	SHP patient's house	Kumamoto, Japan	7 7 5 7 6 4 5 3 2 5			
SHP 12	SHP patient's house	Kumamoto, Japan	7 7 5 7 7 4 5 3 3 5			
SHP 13	SHP patient's house	Kumamoto, Japan	7 7 5 7 6 6 5 3 2 5			
SHP 14	SHP patient's house	Kumamoto, Japan	7 7 5 7 6 6 5 3 2 5			
SHP 15	SHP patient's house	Kumamoto, Japan	7 7 5 7 6 4 5 3 3 7			
SHP 16	SHP patient's house	Kumamoto, Japan	7 7 5 7 6 4 5 3 2 5			
SHP 17	SHP patient's house	Kumamoto, Japan	7 7 5 7 7 4 5 3 3 5			
Environmental isolates[d]						
E.I.1	Soil	Tokyo, Japan	7 7 5 3 7 6 5 2 2 5	0 0 0 0 1 0 0 0 0 0 0 0 1 0 1 0 0 0 0 0 1 0	0 0 1 1 1 1 1 1 0 0	0 0 0 0 1 0 0 0 1 0 1 0 0 0
E.I.2	Soil	Tokyo, Japan	7 7 5 3 7 6 5 2 3 5	0 1 0 0 1 0 0 0 0 0 0 0 1 0 1 0 0 0 0 0 1 0	0 0 0 0 1 0 0 1 0 0	0 0 0 0 0 0 0 0 0 0 1 1 0 1
E.I.3	Soil	Tokyo, Japan	7 7 5 7 7 6 5 2 3 5	0 0 0 0 1 0 0 0 0 0 0 0 1 0 1 0 0 0 1 0 1 0	0 0 1 1 1 1 1 0 0 0	0 0 0 0 1 0 0 0 1 0 1 0 0 0
E.I.4	Soil	Tokyo, Japan	7 7 5 3 7 6 5 2 3 5	0 0 0 0 1 0 0 0 0 0 0 0 1 0 1 0 0 0 0 0 1 0	0 0 0 1 1 1 0 0 1 0	0 0 0 0 0 0 0 0 1 0 1 0 0 0
E.I.5	Soil	Tokyo, Japan	7 7 5 3 7 6 5 2 3 5	0 0 0 1 0 0 1 1 0 0 1 0 0 1 1 0 0 1 0 1 0 0	0 0 0 1 1 0 0 1 1 0	0 0 1 0 1 0 0 0 0 0 1 0 0 0
E.I.6	Soil	Tokyo, Japan	7 7 5 7 6 4 5 3 2 5	0 0 0 1 0 0 0 1 0 0 1 0 0 0 1 0 0 0 0 1 0 0	0 0 0 1 1 0 0 1 1 0	0 0 0 1 0 0 0 1 0 0 0 1 0 0
E.I.7	Soil	Tokyo, Japan	7 7 5 3 7 6 5 2 3 5	0 0 1 0 0 1 0 0 0 1 0 0 1 0 0 1 0 1 0 0 1 0	0 0 0 0 1 0 0 1 0 0	0 0 0 0 0 0 0 0 0 0 0 0 1 0 0
E.I.8	Soil	Tokyo, Japan	7 7 5 3 7 6 5 2 3 5	0 0 1 1 0 1 0 0 0 1 0 1 1 0 0 1 0 1 0 0 1 1	0 1 0 1 1 0 0 1 0 0	0 0 0 0 0 0 0 0 0 0 0 0 1 0 0
E.I.9	Soil	Tokyo, Japan	7 7 5 7 7 6 5 2 3 5	0 0 1 1 0 1 0 0 0 1 0 1 1 0 0 1 0 1 0 0 1 1	0 1 0 1 1 0 0 1 0 0	0 0 0 0 0 0 0 0 0 0 0 0 1 0 1
E.I.10	Soil	Tokyo, Japan	7 7 5 3 7 6 5 2 3 5	0 0 1 1 0 1 0 0 0 1 0 1 1 0 0 1 0 1 0 0 1 1	0 1 0 1 1 0 0 1 0 0	0 0 0 0 0 0 0 0 0 0 0 0 1 0 1
E.I.11	Soil	Tokyo, Japan	7 7 5 3 7 6 5 2 3 5	0 0 1 1 0 1 0 0 0 1 0 0 1 0 0 1 0 1 0 0 1 0	0 0 0 1 1 0 0 1 0 0	0 0 0 0 0 0 0 0 0 0 0 0 1 0 1
E.I.12	Soil	Tokyo, Japan	7 7 5 7 7 6 5 2 3 5	0 0 1 1 0 1 0 0 0 1 0 0 1 0 0 1 0 1 0 0 1 0	0 1 0 1 1 0 0 1 0 0	0 0 0 0 0 0 0 0 0 0 0 0 1 0 1
E.I.13	Soil	Tokyo, Japan	7 7 5 7 7 6 5 2 3 5			
E.I.14	Soil	Tokyo, Japan	7 7 5 3 7 6 5 2 3 5			
E.I.15	Soil	Tokyo, Japan	7 7 5 3 7 6 5 2 3 5			
E.I.16	Soil	Tokyo, Japan	7 7 5 3 7 6 5 2 3 5			
E.I.17	Soil	Tokyo, Japan	7 7 5 3 7 6 5 2 3 5			
E.I.18	Soil	Tokyo, Japan	7 7 5 7 6 6 5 2 2 5			
E.I.19	Soil	Tokyo, Japan	7 7 5 7 6 4 5 2 2 5			
E.I.20	Soil	Tokyo, Japan	7 7 5 3 7 6 5 2 3 5			
E.I.21	Soil	Tokyo, Japan	7 7 5 7 6 4 5 2 2 5			
E.I.22	Soil	Tokyo, Japan	7 7 5 7 7 7 5 3 3 5			
E.I.23	Soil	Tokyo, Japan	7 7 5 3 7 6 5 2 2 5			
E.I.24	Soil	Tokyo, Japan	7 7 5 3 7 6 5 2 3 5			
E.I.25	Soil	Tokyo, Japan	7 7 5 3 7 6 5 2 2 5			
E.I.26	Soil	Tokyo, Japan	7 7 5 3 7 6 5 2 2 5			
E.I.27	Soil	Tokyo, Japan	7 7 5 3 7 6 5 2 2 5			

[a] JU, Juntendo University School of Medicine, Tokyo, Japan; KULM, Kitazato University Laboratory of Mycology, Kanagawa, Japan; KU, Kumamoto University School of Medicine, Kumamoto, Japan; NU, Nagasaki University School of Medicine, Nagasaki, Japan; OMU, Oita Medical University, Oita, Japan; TMBH, Tokyo Metropolitan Bokuto Hospital, Tokyo, Japan; NYSDH, New York State Department of Health, Albany; UMSMT, University of Maryland School of Medical Technology, Baltimore.

[b] 1, PCR product present; 0, fragment absent.

[c] See Kemker et al. (9).

TABLE 2. Specificity of primers M13-7F and M13-7R for *Trichosporon* species and related species

Species	Strain[a]	PCR product[b]
Trichosporon coremiiforme	CBS 2482	—
Trichosporon faecale	CBS 4828	—
Trichosporon asteroides	CBS 2481	—
Trichosporon aquatile	CBS 5973	—
Trichosporon brassicae	CBS 6382	—
Trichosporon cutaneum	CBS 2466	—
Trichosporon dulcitum	CBS 8257	—
Trichosporon inkin	CBS 5585	—
Trichosporon gracile	CBS 8189	—
Trichosporon montevideense	CBS 6721	—
Trichosporon mucoides	CBS 7625	—
Trichosporon ovoides	CBS 5585	—
Trichosporon pullulans	CBS 2532	—
Candida albicans	CBS 562	—
Candida glabrata	IFO 0622	—
Candida guilliermondii	CBS 566	—
Candida kefyr	JCM 9559	—
Candida lusitaniae	CBS 4413	—
Candida parapsilosis	ATCC 22019	—
Candida tropicalis	ATCC 7349	—
Cryptococcus neoformans var. *neoformans*	CBS 132	—
Cryptococcus neoformans var. *gattii*	NIH 191	—
Cryptococcus albidus	CBS 142	—
Malassezia furfur	CBS 1878	—
Rhodotorula mucilaginosa	CBS 17	—

[a] Abbreviations: CBS, Centraal Bureau voor Schimmelcultures, Baarn, The Netherlands; IFO, Institute for Fermentation, Osaka, Japan; JCM, Japan Collection of Microorganisms, Saitama, Japan; NIH, National Institutes for Health, Bethesda, Md.

[b] —, no product obtained.

RAPD analysis. Nuclear DNA was extracted by the method of Makimura et al. (11). Eleven oligonucleotides (15, 21) were preliminarily investigated for reactivity and reproducibility using *T. asahii* DNA: M13 (GAGGGTGGCGGTTCT), T3B (AGGTCGCGGGTTCGAATCC), $(GACA)_4$ (GACAGACAGACAGAC A), TELO1 (TGGGTGTGTGGGTGTGTGGGTGTG), $(CAG)_4$ (CAGCAGC AGCAG), OPE1 (CCCAAGGTCC), OPE2 (GGTGCGGGAA), OPE3 (CC AGATGCAC), OPE4 (GTGACATGCC), R28 (ATGGATCCGC), and RC8 (GGATGTCGAA). Three oligonucleotides, M13, OPE1, and RC8, were selected as single primers for PCR fingerprinting of 39 representative isolates. The three PCRs were individually optimized, and the reaction parameters for each primer were critical. Amplifications were performed in a total buffer volume of 50 μl containing 5 μl of 10× PCR buffer (100 mM Tris-HCl [pH 8.3], 500 mM KCl, 15 mM $MgCl_2$; Nippon Gene, Toyama, Japan), 4 μl of 200 μM deoxynucleoside triphosphates (equimolar dNTPs; Nippon Gene), 30 pmol of each primer, and 2.5 U of Gene *Taq* DNA polymerase (Nippon Gene). Gene *Taq* DNA polymerase was developed for RAPD analysis. For primer M13, PCR was performed in a thermocycler (model 9700; Perkin-Elmer Applied Biosystems, Foster City, Calif.) with an initial denaturation at 94°C for 3 min, followed by 40 cycles of 20 s at 94°C, 60 s at 50°C, and 20 s at 72°C, and a final extension at 72°C for 10 min. For primers OPE1 and RC8, PCR was performed with an initial denaturation at 94°C for 3 min, followed by 40 cycles consisting of 20 s at 94°C, 60 s at 36°C, and 20 s at 72°C, and a final cycle of 10 min at 72°C. Amplification products were separated by 1.5% agarose gel electrophoresis in 1× TAE (Tris-acetate EDTA) buffer. Electrophoretic bands were sized automatically using Digital Science Image Analysis Software (Eastman Kodak, Rochester, N.Y.). Each DNA fragment was scored as present or absent. The intensities of the PCR fragments were not measured. A phenogram showing the similarities of isolates was generated by the unweighted pair group method with arithmetic mean (UPGMA phenogram), based on the pairwise similarity coefficient matrix. The PAUP program (version 4.0b2; Phylogenetic Analysis Using Parsimony; David L. Swofford, Laboratory of Molecular Systematics, National Museum of Natural History, Smithsonian Institution) was used to calculate similarity values and to generate the UPGMA phenogram. A similarity value (S_{AB}) was calculated for each pair of patterns, based on matching fragment positions. Eighteen clinical isolates from eight centers, 9 isolates from SHP patients' houses, and 12 environmental isolates were analyzed by RAPD analysis.

Origin-specific DNA sequences from RAPD fingerprinting. Forty-six polymorphic bands were used to determine whether there was an origin-specific DNA band. Although three origin-specific DNA bands were not found, we observed a specific 330-bp band (see Fig. 1) for the clinical isolates and the strains obtained from the homes of SHP patients in RAPD fingerprinting using primer M13. The 330-bp DNA fragment was extracted from an agarose gel using a NucleoSpin kit (Clontech Laboratories Inc., Palo Alto, Calif.) according to the manufacturer's instructions. The fragment was cloned into pCR-2.1 using a TA cloning kit (Invitrogen Corp., Carlsbad, Calif.) and was sequenced with an ABI PRISM Cycle-Sequencing kit (Applied Biosystems). From these DNA sequences, two oligonucleotide primers were designed: M13-7F (TGCGCTCATGCGCTCATG AC) and M13-7R (TCCGCTGAGGAAGGAAGAGC). The PCR conditions were as follows: initial denaturation at 94°C for 3 min, followed by 30 cycles of 20 s at 94°C, 60 s at 50°C, and 20 s at 72°C, and a final cycle of 10 min at 72°C. For this PCR, Takara (Shiga, Japan) Ex *Taq* polymerase was used. Table 2 shows the strains used for specificity of PCR.

Biochemical characteristics. The isolates were examined with an ID32C kit (bioMérieux SA, Marcy l'Etoile, France) in accordance with the manufacturer's instructions. A total of 42 clinical isolates, 17 SHP isolates, and 27 environmental isolates were examined. The PAUP program was used to calculate similarity values and to construct a UPGMA phenogram.

Nucleotide sequence accession number. The sequences of specific DNA fragments obtained by PCR of *T. asahii* clinical isolates and strains isolated from SHP patients' houses have been deposited in the DNA Data Bank of Japan under accession number AB049759.

RESULTS

RAPD analysis. The three primers produced 46 polymorphic bands, as shown in Table 1. A representative photograph of the PCR products for primer M13 is presented in Fig. 1. The similarities of the polymorphic bands within the clinical isolates, strains isolated from SHP patients' houses, and environmental isolates were 91.3, 74.5, and 72.0%, respectively (Table 3). Clinical isolates were more similar to each other than to the strains of the two other origins ($P < 0.01$). and they formed two clusters (Fig. 2). Although the clinical isolates were obtained from eight different Japanese and U.S. centers, there was no relationship between the RAPD fingerprinting pattern and the

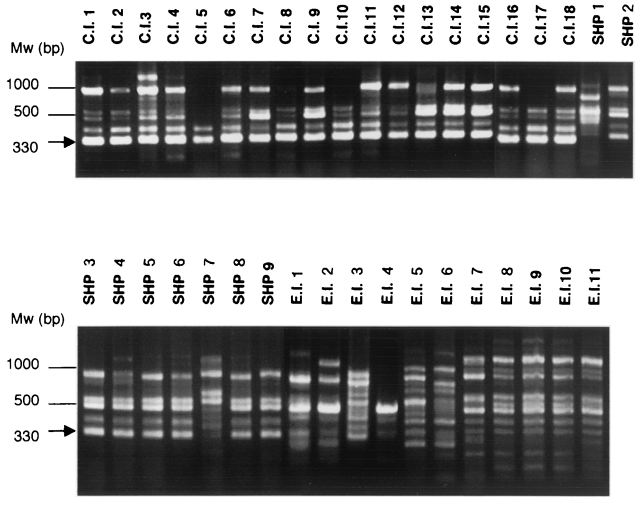

FIG. 1. Representative electrophoresis gel of PCR fingerprints obtained from *T. asahii* isolates using primer M13. Mw, molecular weight marker. A 330-bp DNA fragment (arrow) shows specificity for the clinical isolates and strains isolated from SHP patients' houses.

TABLE 3. Mean similarity between pairs of *T. asahii* isolates from three different origins

Comparison	Mean similarity (%) ± SD
Within clinical isolates	91.3 ± 4.5
Within strains isolated from SHP patients' houses	74.5 ± 12.5
Within environmental isolates	72.0 ± 14.9
Between clinical isolates and strains isolated from SHP patients' houses[a]	78.0 ± 12.0
Between clinical isolates and environmental isolates[a]	66.9 ± 13.7
Between strains isolated from SHP patients' houses and environmental isolates[b]	68.9 ± 12.4

[a] Significant difference ($P < 0.01$).
[b] No significant difference ($P > 0.1$).

source hospital. Neither environmental isolates nor strains isolated from SHP patients' houses formed a cluster ($P > 0.1$).

Origin-specific DNA fragment. An origin-specific 330-bp DNA fragment was obtained. Newly designed oligonucleotide primers amplified all *T. asahii* clinical isolates. PCRs were positive for the strains isolated from SHP patients' houses, with the exception of strain SHP 1. DNA from environmental isolates, excluding strain E.I.3, was amplified by PCR and was

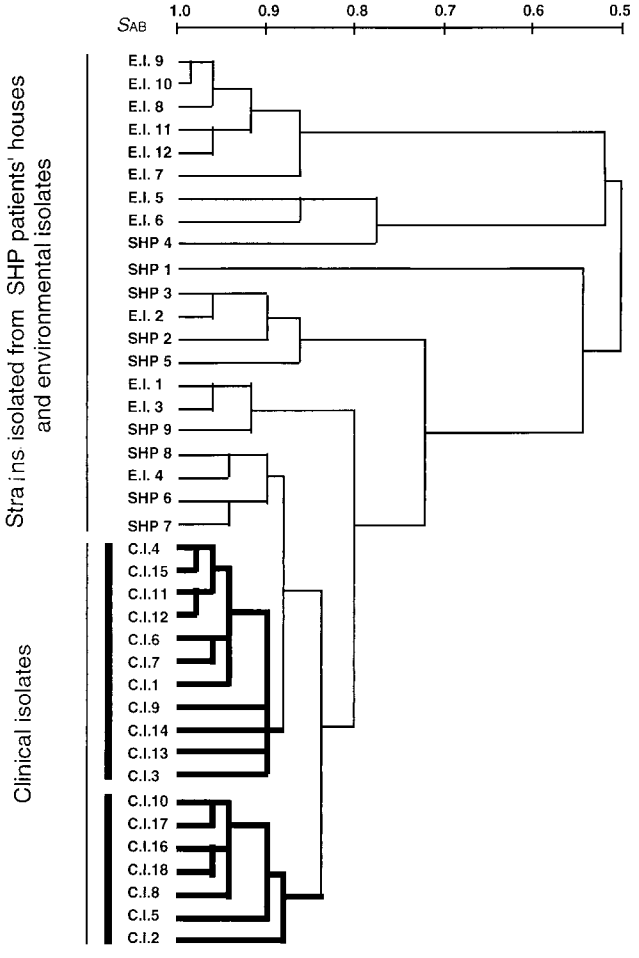

FIG. 2. UPGMA phenogram of *T. asahii* isolates calculated from DNA fingerprinting patterns obtained with primers M13, OPE1, and RC8.

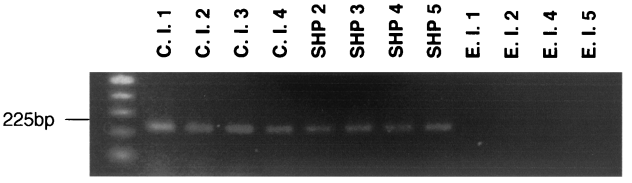

FIG. 3. Representative electrophoretic gel of PCR products using primers M13-7F and M13-7R. A 225-bp PCR product was amplified.

negative for all other *Trichosporon* species and other medically relevant yeasts (Table 2). A representative electrophoretic gel of the PCR product is shown in Fig. 3. The sequences obtained from specific DNA fragments have been deposited in the DNA Data Bank of Japan (accession number AB049759).

Biochemical characteristics determined by using the ID32 kit. The biochemical characteristics of the isolates are listed as an API profile in Table 1. The phenogram shown in Fig. 4 was generated using 31 characteristics and consists of three major clusters: I, II, and III. Cluster I includes most of the clinical and SHP strains (93%; 55 of 59), and cluster II comprises most of the environmental isolates (85%; 23 of 27). Cluster III contains only one strain, C.I.28. The ability to utilize 25 of 31 compounds was almost the same for strains belonging to clusters I and II; however, remarkable differences were found in the ability to utilize the other 6 compounds, as shown in Table 4.

DISCUSSION

Because *T. asahii* is responsible for both opportunistic fungal infections and allergies and is also distributed in the environment, it is interesting to examine the genetic diversity of strains obtained from different sources. RAPD analysis suggested that the clinical isolates were distinct from isolates obtained from SHP patients' houses and the environmental isolates. While the assimilation patterns of clinical isolates and strains obtained from SHP patients' houses were similar, they were notably different from those of environmental isolates. Comparison of Fig. 2 and 4 shows that the figures are not entirely correlated with each other in the phenogram. However, it is obvious that the clinical and environmental isolates have distinct RAPD profiles and biochemical characteristics. A similar finding was reported by Bertout et al. (3), who found that *C. neoformans* isolates formed three clusters in an MLEE data analysis. The first cluster contained clinical isolates, the second included environmental isolates, and the third contained strains isolated from either patients or the environment. In 1991, blood, superficial, and environmental isolates of *T. beigelii* were reported to be distinct, based on RFLP of rDNA and isoenzyme profiles (9). Subsequently, *T. beigelii* has been divided into more than 10 species, and some of the superficial and environmental isolates have been reidentified as *Trichosporon aquatile, T. cutaneum, T. domesticum*, and *T. ovoides* according to current taxonomical criteria (16; see also the American Type Culture Collection [ATCC, Rockville, Md.] catalog at http://www.atcc.org/). In addition to the correlation between the genotype and the origin of a strain, RAPD can be used to differentiate genotype and drug susceptibility. Xu et al. (21) showed that fluconazole-resistant *C. albicans* strains isolated from patients infected with human immunodeficiency virus

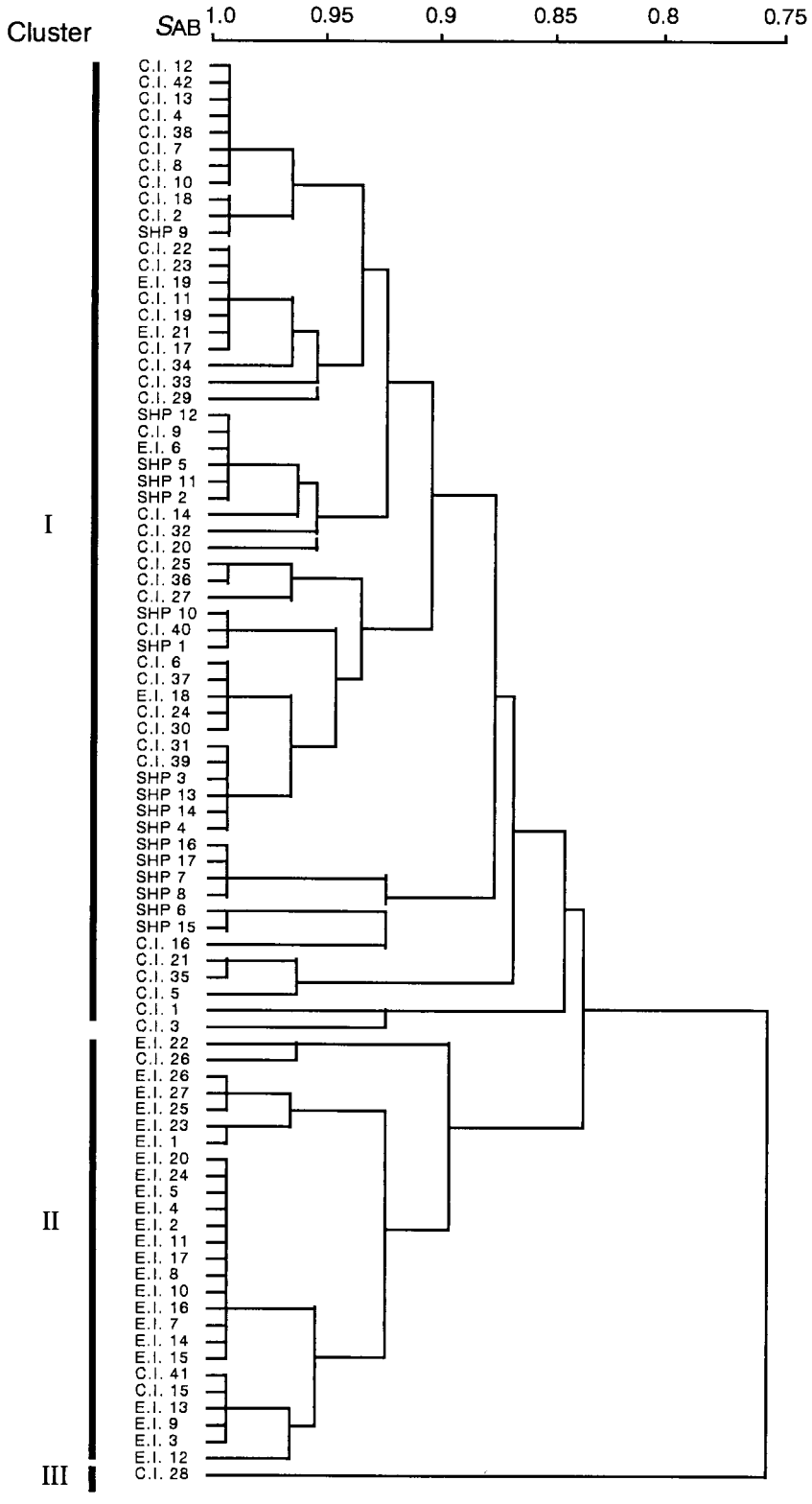

FIG. 4. UPGMA phenogram of *T. asahii* isolates calculated from the ability to utilize 31 compounds.

formed a cluster distinct from that of fluconazole-sensitive strains by RAPD. Strains C.I.16, C.I.17, and C.I.18 were resistant to fluconazole (data not shown). We have not yet determined the drug susceptibilities of all *T. asahii* isolates, but they were in the same subcluster. No correlation between the RAPD fingerprinting pattern and the hospital was found for *T. asahii* isolates, whereas for *C. albicans*, there is hospital, regional, and country specificity among isolates. In this study,

TABLE 4. Characteristics differentiating strains belonging to clusters I and II

Cluster	% of strains able to utilize the indicated compound[a]					
	MDG	SOR	RHA	MLZ	SBE	MAN
I (n = 59)	100	14	41	56	29	14
II (n = 26)	31	100	100	4	0	81

[a] MDG, α-methyl-D-glucoside; SOR, sorbitol; RHA, rhamnose; MLZ, melezitose; SBE, sorbose; MAN, mannitol.

11 oligonucleotides that had been widely used for *C. albicans* and *C. neoformans* were tested against *T. asahii* DNA. Of these 11 oligonucleotides, PCR parameters could not be optimized for 8. The development of a highly or specifically reactive oligonucleotide primer would permit an intensive epidemiological study of disease due to *T. asahii*.

In this study, we obtained DNA fragments specific for clinical isolates and strains isolated from SHP patients' houses. We previously reported species-specific DNA sequences derived from *T. asahii* by ITS sequence analysis (17, 18). Since *Trichosporon* species are phylogenetically closely related, it is difficult to design species-specific primers. Our *T. asahii*-specific primers designed from ITS-derived sequences amplify DNA of *T. faecale* (*Trichosporon asahii* var. *faecalis*), and *T. coremiiforme* (*Trichosporon asahii* var. *coremiiformis*). The latter two species are nonpathogenic and are not known to cause clinical problems. If new oligonucleotide primers are designed, it should be possible to detect *T. asahii* with high specificity. No similar sequence was found in GenBank by a BLAST search (http://www.ncbi.nlm.nih.gov/BLAST/).

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