Molecular phylogenetic study of the basidiomycetous anamorphic yeast genus *Trichosporon* and related taxa based on small subunit ribosomal DNA sequences

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Accepted for publication 27 December 1997

Small subunit ribosomal DNA sequences of all species of the basidiomycetous anamorphic yeast genus *Trichosporon* were determined, and phylogenetic trees were constructed by the neighbor-joining and maximum likelihood methods. The sequence data showed that, with the exception of *T. pullulans*, the genus is monophyletic, although its members have two different major ubiquinones, Q9 and Q10. The genus can be divided phylogenetically into three major clusters. Species with Q10 as the major ubiquinone constitute a single cluster, while those with Q9 form two clusters. *Trichosporon pullulans* was phylogenetically distinct from other taxa of the genus. It is located in a cluster containing *Cystofilobasidium capitatum*, *Mrakia frigida*, *Xanthophyllomyces dendrorhous* and three species of *Udeniomyces*. This result suggests that *T. pullulans* does not belong to the genus *Trichosporon*.

Key Words——phylogeny; SSU rDNA; *Trichosporon*.

Trichosporon Behrend is a genus of basidiomycetous anamorphic yeast. Its key taxonomic characters are: production of arthrospores, absence of alcoholic fermentation, possession of Q9 or Q10 as a major ubiquinone component, and the presence of xylose in the cell wall (Kreger-van Rij, 1984; Weijman, 1979). Among the species of the genus *Trichosporon*, several yeast taxonomists have pointed out that *T. cutaneum* is heterogeneous on the basis of ubiquinone type, DNA relatedness, morphology and immunoelectrophoresis pattern of cytoplasmic antigens, and restriction fragment length polymorphism of ribosomal DNA (Guého et al., 1984; Hara et al., 1989; Kemker et al., 1991; Lee et al., 1990; Saito et al., 1985; Yamada et al., 1982). As *T. beigelii* was formerly regarded as conspecific with *T. cutaneum*, the priority of their nomenclature has been discussed (Guého et al., 1992a; Kreger-van Rij, 1984; McPartland and Goff, 1991). At present, the name *T. cutaneum* has priority (Guého et al., 1992a). In 1992, Guého et al. (1992b) reclassified the members of the genus on the basis of physiology, ubiquinone type, DNA relatedness and partial sequences of large subunit ribosomal RNA (LSU rRNA). In 1995, Sugita et al. (1995b) described a new species, *T. domesticum*, which was isolated from a damp and rotten wooden sideboard in a house. At present, 17 species and five varieties are accepted as members of the genus.

Small and/or large subunit rRNA sequences have been extensively used to study phylogenetic relationships among organisms. Several researchers reported recently that the basidiomycetes were divided into three major lineages by analyses of ribosomal RNA sequences (Sugiyama and Suh, 1993; Suh and Nakase, 1995; Suh and Sugiyama, 1994; Swann and Taylor, 1995). The first major lineage contains smut fungi, represented by *Ustilago* and *Tilletia*. The second includes urediniomycetous fungi, including yeasts such as *Sporobolomyces* and *Sporidiobolus*. The third is composed of hymenomycetous yeasts. A species of *Trichosporon*, *T. cutaneum*, is included in the third lineage.

Guého et al. (1989) determined the partial sequences of small subunit (SSU) and LSU rRNA of *T. cutaneum* and *T. pullulans* and indicated that these two species were not closely related phylogenetically. Several researchers analyzed the SSU rDNA of several species of *Bullera* and found that they constituted a cluster with species of *T. cutaneum* (Suh and Nakase, 1995; Suh and Sugiyama, 1993; van der Peer et al., 1992). Guého et al. (1992b) constructed a phylogenetic tree based on partial sequences of LSU rRNA and indicated that this genus could be divided into five major groups.

In this study, we determined the sequences of SSU rDNA of all species in the genus *Trichosporon* and analyzed the phylogenetic relationship among them and related basidiomycetous yeasts.

Materials and Methods

Strains used The 17 species and five varieties belonging to the genus *Trichosporon* that were used for sequencing are shown in Table 1 with their DDBJ accession numbers of the sequence data of SSU rDNA. All are the type strains of their respective taxa.

Sequencing of SSU rDNA DNA was obtained from cells by heating them in a lysing solution containing a detergent (Makimura et al., 1994). The SSU rRNA coding region was amplified by the polymerase chain reaction (PCR) as described in Suh and Nakase (1995). The PCR products were sequenced by using a SequiTherm Long-Read Cycle Sequencing Kit (Epicentre Technologies, Wisconsin, U.S.A.) with the primer as described in Suh et al. (1996). DNA sequence reactions were analyzed with an ALF*red* DNA sequencer (Pharmacia Biotech, Uppsala, Sweden).

Phylogenetic analysis The sequences were aligned using the Clustal W computer program (Thompson et al., 1994). For phylogenetic analysis, the following reference SSU rDNA sequences were obtained from the nucleotide sequence libraries (EMBL, GenBank and DDBJ): *Athelia bombacina* (M55638), *Auricularia auricula-judae* (L22254), *A. polytricha* (L22255); *Boletus santana* (M94337), *Bullera dendrophila* (D31649), *Bullera miyagiana* (D31651), *Bulleromyces albus* (X60179), *Calocera cornea* (L22256), *Coprinus cinereus* (M92991), *Cystofilobasidium capitatum* (D12801), *Cryptococcus albidus* (D31655), *Dacrymyces chrysospermus* (L22257), *Dacrymyces stillatus* (L22258), *Erythrobasidium hasegawianum* (D12801), *Fellomyces polyborus* (D64117), *Fibulobasidium inconspicuum* (D64123), *Filobasidiella neoformans* (X60180), *Filobasidium floriforme* (D13460), *Heterotextus alpinus* (L22259), *Kockovaella thailandica* (D64133), *Mrakia frigida* (D12802), *Rhodosporidium toruloides* (D12806), *Spongipellis unicolor* (M59760), *Sporidiobolus johnsonii* (L22261), *Sporobolomyces roseus* (X60181), *Sterigmatosporidium polymorphum* (D64120), *Thanatephorus praticola* (M92990), *Tilletia caries* (U00972), *Tremella moriformis* (U00977), *T. globospora* (U00976), *T. foliacea* (L22262); *Tsuchiyaea wingfieldii* (D64121), *Udeniomyces megalosporus* (D31657), *U. piricola* (D31659), *U. puniceus* (D31658); *Ustilago hordei* (U00973), *U. maydis* (X62396); *Xanthophyllomyces dendrorhous* (D31656), *Xerocomus chrysenteron* (M94340). The ascomycetous yeasts *Saccharomyces cerevisiae* (J01353) and *Candida albicans* (X53497) and the filamentous ascomycete *Neurospora crassa* (X04971) were used as outgroups. For the analysis using the neighbor-joining method (Saito and Nei, 1987), distances between the sequences were calculated using Kimura's two-parameter model (Kimura, 1980). Sites where gaps existed in any of the sequences were excluded. The DNAML program in the PHYLIP 3.5c package (distributed by J. Felsenstein, Univ. Washington) was used for maximum likelihood analysis with a transition/transversion ratio of PHYLIP of 2.000000. Boot-

Table 1. Strains used in this study.

Species	Strain[a]	DDBJ accession number[b]
Trichosporon aquatile	JCM 3936[c]	AB001730
Trichosporon asahii var. *asahii*	JCM 2389[c]	AB001726
Trichosporon asahii var. *coremiformis*	JCM 2938[c]	AB001727
Trichosporon asahii var. *faecalis*	JCM 2941[c]	AB001728
Trichosporon asteroides	JCM 2937[c]	AB001729
Trichosporon brassicae	JCM 1599[c]	AB001731
Trichosporon cutaneum	JCM 1462[c]	AB001753
Trichosporon domesticum	JCM 9580[c]	AB001754
Trichosporon dulcitum	JCM 10017[c]	AB001755
Trichosporon gracile	JCM 10018[c]	AB001756
Trichosporon inkin	JCM 9195[c]	AB001757
Trichosporon jirovecii	JCM 9935[c]	AB001758
Trichosporon loubieri var. *loubieri*	JCM 2947[c]	AB001759
Trichosporon loubieri var. *laibachii*	JCM 3939[c]	AB001760
Trichosporon loubieri var. *laibachii* (=*Trichosporon multisporum*)	JCM 9934	AB001764
Trichosporon moniliiforme	JCM 2389[c]	AB001761
Trichosporon montevideense	JCM 9937[c]	AB001762
Trichosporon mucoides	JCM 9939[c]	AB001763
Trichosporon ovoides	JCM 9940[c]	AB001765
Trichosporon pullulans	JCM 9886[c]	AB001766
Trichosporon sporotrichoides	JCM 9941[c]	AB001767

a) JCM, Japan Collection of Microorganisms, The Institute of Physical and Chemical Research (RIKEN), Saitama, Japan.
b) DDBJ, DNA Data Bank of Japan, National Institute of Genetics, Shizuoka, Japan.
c) Type strain.

strap analysis (Felsenstein, 1985) was performed from 1,000 random resamplings.

Results and Discussion

The SSU rDNA sequences of the 21 strains determined in this work were aligned with other sequences including those of related basidiomycetous yeasts. The phylogenetic trees were constructed by neighbor-joining (Fig. 1) and maximum likelihood methods (Fig. 2). Sites where gaps existed in any of the sequences were excluded, and 1,590 nucleotides were used in this analysis. As shown in Fig. 1, the basidiomycetes were divided into three major lineages, 1, 2 and 3, based on phylogenetic analyses, as reported by several researchers (Sugiyama and Suh, 1993; Suh and Nakase, 1995; Suh and Sugiyama, 1994; Swann and Taylor, 1995). The first lineage has the smut fungi (Ustilaginales), *Ustilago* and *Tilletia*. The remaining two lineages correlated well with the presence or absence of xylose in the cell walls. Species of the second lineage lack xylose, but those of the third contain it. Recently, Suh et al. (1996) reported that cluster 3 could be divided into two subclusters, 3a and 3b. All species of the genus *Trichosporon* sequenced in this study were located in cluster 3. *Trichosporon pullulans* was located in subcluster 3a and the remaining species were located in subcluster 3b. The 16 species and five varieties in subcluster 3b were monophyletic with 99.9% bootstrap confidence level in the tree. These species could be divided phylogenetically into three major groups, I, II and III. These groups clearly correspond to the major ubiquinone types, Q9 and Q10. Our phylogenetic tree based on SSU rDNA sequences differs slightly from that based on partial sequences of LSU rRNA that has been reported (Guého et al., 1992b), but the topology of the two trees is almost identical. Group I was composed of the species possessing Q10, *T. cutaneum*, *T. jirovecii*, *T. moniliiforme* and *T. mucoides*. Groups II and III were composed of the species having Q9.

Group II included five species and three varieties: *T. asahii* var. *asahii*, *T. asahii* var. *coremiformis*, *T. asahii* var. *faecalis*, *T. asteroides*, *T. aquatile*, *T. inkin* and *T. ovoides*. The latter is the type species of the genus. Three varieties of *T. asahii* were described based on their intermediate DNA relatedness (Sugita et al., 1994), which has been confirmed using clinical isolates (Sugita et al., 1995a). They were closely related on the tree. Group III contained seven species and two varieties: *T. brassicae*, *T. domesticum*, *T. dulcitum*, *T. gracile*, *T. loubieri* var. *laibachii*, *T. loubieri* var. *loubieri*, *T. montevideense*, and *T. sporotrichoides*. Two varieties of *T. loubieri* formed a cluster with 96.8% bootstrap confidence level. Guého et al. (1992a) found that *T. loubieri* var. *laibachii* JCM 9934 (type strain of *T. multisporum*) was close to the type strain of *T. loubieri* var. *laibachii* on the basis of partial sequences of LSU rRNA, but that they differed significantly in the mol% G+C. These two strains also showed low relatedness to each other in a DNA-DNA hybridization experiment. Guého et al. (1992a) noted that the taxonomic position of *T. loubieri* var. *laibachii* CBS 2495 (=JCM 9934) has remained uncertain due to the difficulty of DNA purification from this strain. Phylogenetically, *T. loubieri* var. *laibachii* JCM 9934 showed a close relationship with the type strains of two varieties of *T. loubieri*. *Trichosporon sporotrichoides* was included in group III in the neighbor-joining tree but was separated from the other species of the genus *Trichosporon* in the maximum likelihood tree.

Trichosporon pullulans was not closely related to any other *Trichosporon* species but was located in a cluster that contained *Cystofilobasidium capitatum*, *Mrakia frigida*, *Xanthophyllomyces dendrorhous* and three species of *Udeniomyces*. Some researchers have suggested that *T. pullulans* is distinct from other *Trichosporon* species on the basis of assimilation of nitrate and partial sequences of LSU rDNA, despite the fact that *T. pullulans* produces arthrospores abundantly (Fell et al., 1995; Guého et al., 1992b, 1993). Our sequence data of SSU rDNA strongly suggest that *T. pullulans* does not belong to the genus *Trichosporon*. It appears reasonable to transfer *T. pullulans* to a distinct genus from the genus *Trichosporon*. Further taxonomic and phylogenetic studies are needed to clarify the rational position of *T. pullulans*. Although *T. pullulans* formed a cluster with *C. capitatum*, *M. frigida*, *X. dendrorhous* and the three species of *Udeniomyces*, its ubiquinone type is different from other yeast species. *Trichosporon pullulans* has Q9 as the major component, while the other species have Q8 or Q10.

Serological researches have also been done in the genus *Trichosporon*. Ikeda et al. (1996) and Nishiura et al. (1997) revealed that *Trichosporon* species have at least three serological types, I, II, III. All of the species of the genus *Trichosporon* were serotyped by using their specific factor sera. Our molecular analysis data based on SSU rDNA sequences correlated well with the serological grouping. Phylogenetic groups I and II of the genus *Trichosporon* corresponded to serotypes I and II, respectively. Species of group III showed serotypes III or I–III (type I–III reacts with both factor sera I and III). In phylogenetic group III, species that corresponded to serotype III were: *T. brassicae*, *T. domesticum* and *T. montevideense*. They made branches with 98.8 to 100% bootstrap confidence level. *Trichosporon pullulans* did not react with any factor sera.

Historically, some species in the genus *Trichosporon* have been known as causative agents of white piedra, an innocuous hair shaft infection encountered mostly in tropical and temperate countries. The species has subsequently emerged as an infrequent but often fatal opportunistic pathogen in immunocompromised patients (Nahass et al., 1993; Walsh, 1989). Taxonomical investigation of the causative agents of trichosporonosis has been conducted by Guého et al. (1994), Herbrecht et al. (1993) and Sugita et al. (1995b, 1996). They reported seven human pathogenic species: *T. asahii*, *T. asteroides*, *T. cutaneum*, *T. domesticum*, *T. inkin*, *T. mucoides* and *T. ovoides*. The majority of these agents were found to belong to serotype II group. All species

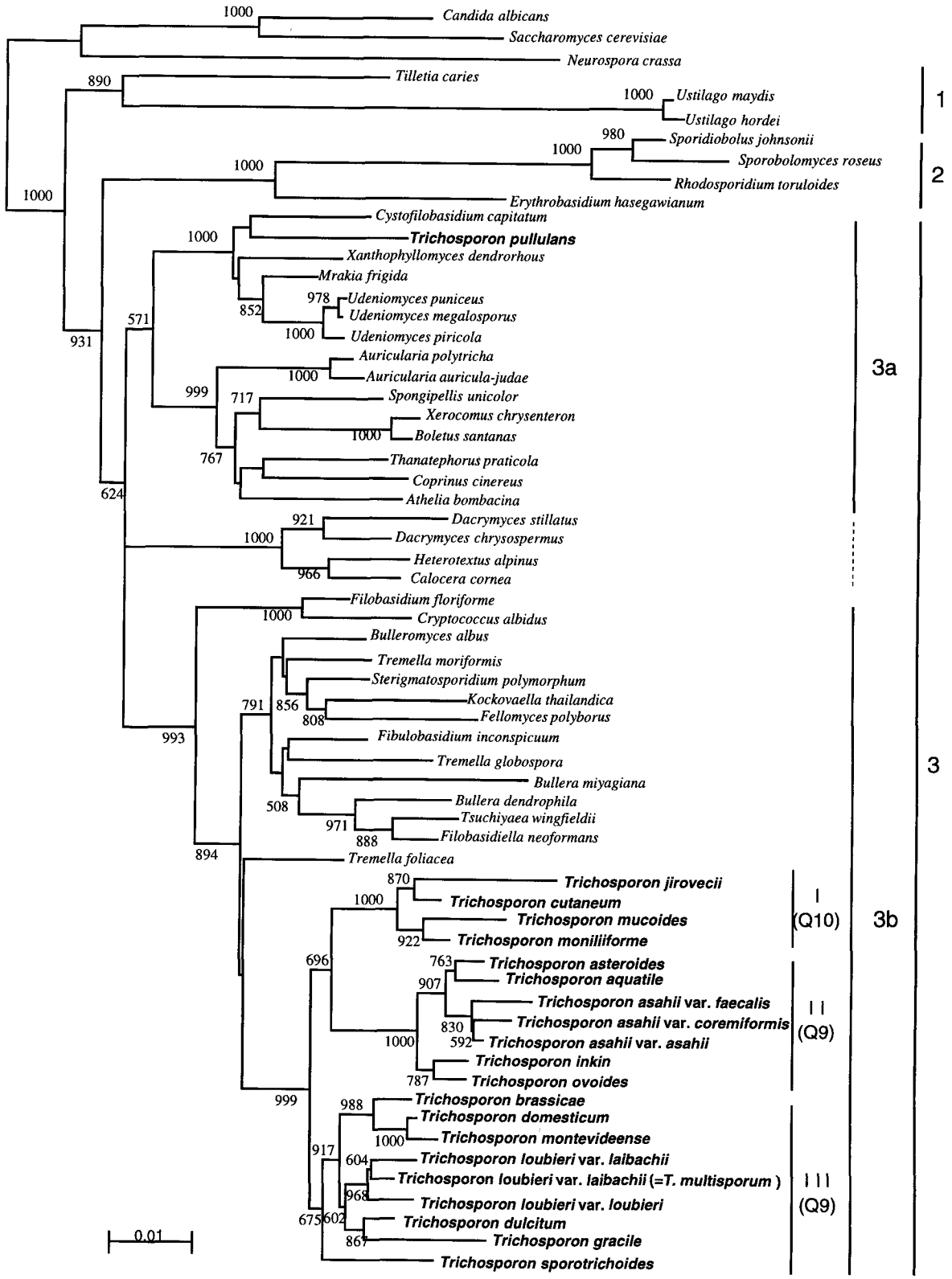

Fig. 1. Phylogenetic tree based on SSU rDNA sequences for the *Trichosporon* species and related taxa. The tree was constructed by the neighbor-joining method. The numerals represent the confidence level from 1,000 replicate bootstrap samplings (frequencies less than 50% are not indicated). The distance corresponding to one base change per hundred nucleotide positions is indicated by a bar.

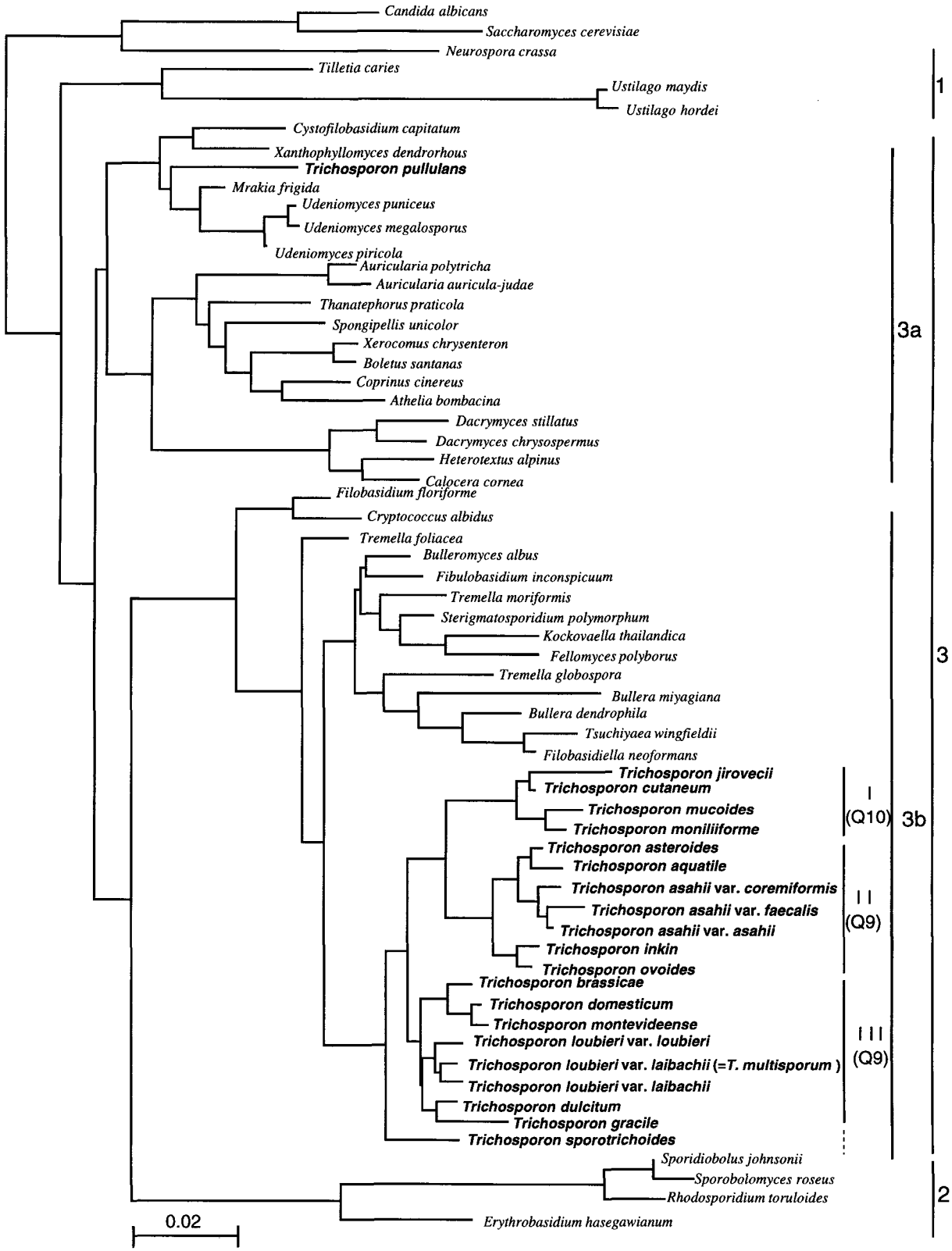

Fig. 2. Phylogenetic tree based on SSU rDNA sequences for the *Trichosporon* species and related taxa. The tree was constructed by the maximum likelihood method.

except *T. aquatile* in the serotype II group were responsible for trichosporonosis. No report has been found indicating that a member of serotype I–III, such as *T. gracile* and *T. loubieri*, was isolated from trichosporonosis patients and that these species might not be responsible for infection. Pathogenic species of *Trichosporon* might be phylogenetically different from non-pathogenic species, in addition to the serological differences.

In conclusion, we have examined the molecular phylogeny of all species of the genus *Trichosporon* based on the sequences of SSU rDNA. Our results suggest that this genus, with the exception of *T. pullulans*, shows monophylety. Revision of the taxonomy of *T. pullulans* will be required in the near future.

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