International Journal of Systematic and Evolutionary Microbiology (2004), **54**, 975–986 DOI 10.1099/ijs.0.02859-0

Systematics of the anamorphic basidiomycetous yeast genus *Trichosporon* Behrend with the description of five novel species: *Trichosporon vadense*, *T. smithiae*, *T. dehoogii*, *T. scarabaeorum* and *T. gamsii*

Wouter J. Middelhoven,[1] Gloria Scorzetti[2] and Jack W. Fell[2]

[1]Laboratorium voor Microbiologie, Wageningen University, Hesselink van Suchtelenweg 4, 6703 CT Wageningen, the Netherlands

[2]Rosenstiel School of Marine and Atmospheric Sciences, University of Miami, 4600 Rickenbacker Causeway, Key Biscayne, Florida 33149, USA

Correspondence
Wouter J. Middelhoven
Wout.Middelhoven@wur.nl

Phylogenetic trees of the anamorphic basidiomycetous yeast genus *Trichosporon* Behrend, based on molecular sequence analysis of the internal transcribed spacer region and the D1/D2 region of the large subunit of ribosomal (26S) DNA, are presented. This study includes three novel species from soils, *Trichosporon vadense* sp. nov. (type strain, CBS 8901^T), *Trichosporon smithiae* sp. nov. (type strain, CBS 8370^T) and *Trichosporon gamsii* sp. nov. (type strain, CBS 8245^T), one novel species from an insect, *Trichosporon scarabaeorum* sp. nov. (type strain, CBS 5601^T) and one species of unknown origin, *Trichosporon dehoogii* sp. nov. (type strain, CBS 8686^T). The phylogenetic positions and physiological characteristics that distinguish the new taxa from related species, based partly on growth tests that are not traditionally used in yeast taxonomy (uric acid, ethylamine, L-4-hydroxyproline, tyramine and L-phenylalanine as sources of carbon and nitrogen, and polygalacturonate, quinate, 4-ethylphenol, phloroglucinol, 2,3-dihydroxybenzoate and orcinol as sole carbon sources), are discussed. Assimilation of L-rhamnose and erythritol and maximum growth temperature were also used to delineate species.

INTRODUCTION

Trichosporon Behrend is a genus of anamorphic yeasts (Basidiomycota, Hymenomycetes, Tremelloidae, Trichosporonales) with distinct morphological characteristics of budding yeast cells and true mycelia that disarticulate to form arthroconidia. The genus consists of soil- and water-associated species, although some species are causative agents of diseases in man and cattle (Kwon-Chung & Bennett, 1992; Guého *et al.*, 1998; de Hoog *et al.*, 2000). With the exception of *Trichosporon pullulans*, the genus is monophyletic as viewed by small-subunit (SSU) rDNA (Guého *et al.*, 1992; Sugita & Nakase, 1998), D1/D2 region of the large-subunit (LSU) rDNA (Guého *et al.*, 1998; Fell *et al.*, 2000) and internal transcribed spacer (ITS) region (Sugita *et al.*, 1999; Scorzetti *et al.*, 2002) sequence analyses. Guého *et al.* (1998), in a review of the genus, accepted 19 species. Sugita & Nakase (1998), based on SSU rDNA analysis of type strains, reported that the genus could be divided into three major groups of distinct species that can be distinguished by serotyping.

The standard technique for species identification of yeasts is based on phenotypic characteristics, including assimilation of about 50 carbon compounds and 10 nitrogen compounds. A major difficulty in obtaining accurate identification of basidiomycetous yeasts is the fact that different strains within a species respond differently to many of these tests. Consequently, strains can be misidentified and the existence of genetically distinct species can be overlooked. This difficulty can be overcome by nuclear base sequencing of rDNA.

Most of the physiological characteristics that are used

Published online ahead of print on 21 November 2003 as DOI 10.1099/ijs.0.02859-0.

Abbreviations: IGS, intergenic spacer; ITS, internal transcribed spacer; LSU, large-subunit; SSU, small-subunit.

The GenBank/EMBL/DDBJ accession numbers for the ITS region sequences of strains CBS 8901^T, CBS 8370^T, CBS 8686^T, CBS 5601^T and CBS 8245^T are AY093425, AF444397, AF444476, AF444446 and AF444424, respectively. The accession numbers for their D1/D2 region sequences are AY093426, AF444706, AF444718, AF444710 and AF444708, respectively.

A phylogenetic tree based on sequencing of the D1/D2 region and a dichotomous identification key to 36 *Trichosporon* species are available as supplementary material in IJSEM Online.

Downloaded from www.microbiologyresearch.org by
IP: 93.91.26.97
On: Fri, 27 Nov 2015 09:58:33

in standard yeast descriptions depend on the activity of specific permeases, hydrolases or dehydrogenases. For most of the carbohydrates and polyalcohols tested, presence of these enzymes links these carbon compounds to central metabolism. In contrast, assimilation of uric acid and phenolic compounds requires the activity of more complex catabolic and anabolic pathways. As some of these compounds are found exclusively in specific environments, their assimilation may give information on the ecology of yeast species. These considerations inspired us to study growth responses to uric acid, ethylamine, polygalacturonate and some phenolic compounds for the distinction of *Trichosporon* species, as an alternative to traditional methods and base sequencing. Previous studies have demonstrated species-specific responses to these compounds (Middelhoven, 1993; Middelhoven *et al.*, 1985, 1999, 2000, 2001; Sampaio, 1999). In some ascomycetous yeasts, growth on such compounds confirmed their phylogenetic relationships (Middelhoven & Kurtzman, 2003). In the present study, we report growth responses to these compounds by strains of all currently accepted species of the genus *Trichosporon*. An identification key, based on these growth tests, assimilation of erythritol and L-rhamnose and maximum growth temperature, is available as supplementary material in IJSEM Online. The present study proposes five novel species in the genus *Trichosporon*, based on nuclear base sequencing and traditional and non-traditional growth tests.

METHODS

Source and characterization of strains. Strains studied are listed in Table 1. Strains of the novel species were examined for morphological and physiological properties by using standard yeast identification methods (Yarrow, 1998). Utilization of carbon sources (0·5 %) was tested in liquid Difco yeast nitrogen base (YNB) at 25 °C on a rotary shaker at 100 r.p.m. The pH of growth media was adjusted to 5·5 as required; however, the pH of media with galacturonic acid or quinic acid was not adjusted, which is in agreement with the laboratory practice of CBS Delft (D. Yarrow, personal communication; Middelhoven, 1997). D-Glucaric (saccharic), L-malic, galactaric (mucic, 0·25 %) and tartaric acids were tested at pH 4·0. Assimilation of nitrogen sources (40 mM assimilable nitrogen) was tested in liquid yeast carbon base (YCB) except for sodium nitrite, which was tested by the auxanographic technique.

Basal growth medium (Middelhoven *et al.*, 1991) that was used for non-traditional growth tests had the composition of YNB, but the phosphate concentration was tenfold higher in order to improve the buffering capacity. The nitrogen source, when present, was 2 g ammonium chloride l^{-1}, rather than 5 g ammonium sulphate l^{-1}, as used in YNB. A droplet of a pre-culture in 2 ml glucose (5 g l^{-1}) basal growth medium was inoculated into 2 ml medium in culture tubes of 150 × 15 mm, which were then incubated on a rotary shaker at 25 °C. No ammonium chloride was added to growth media with nitrogenous substrates, such as uric acid, ethylamine, L-phenylalanine and L-4-hydroxyproline. Uric acid (10 g l^{-1}, 20 mg per tube, pH not adjusted) was tested as the sole source of carbon and nitrogen. Tubes were agitated at 25 °C to keep the crystals in suspension. Sparse but discernible growth at the expense of this energy-poor and weakly soluble substrate was assessed after disappearance of the crystals. Growth on the potentially toxic phenolic compounds 4-ethylphenol, orcinol, phloroglucinol, 2,3-dihydroxybenzoate and tyramine was studied in slant cultures (Middelhoven *et al.*, 1991). Slants of basal growth medium (20 g agar l^{-1}, 7 ml per culture tube) were dried overnight and the agar surface was inoculated with the pre-culture. Substrates (7 mg in 0·1 ml) were added to the bottom of the slant, avoiding contact with the agar surface as much as possible. Substrates reached the cells by diffusion. Assimilation was deduced from a zone of growth higher or lower in the tube, depending on the substrate's toxicity.

Sequence analysis. ITS and D1/D2 rDNA molecular sequencing and analyses were performed at the University of Miami by using procedures described previously (Fell *et al.*, 2000; Scorzetti *et al.*, 2002). Phylogenetic analysis employed PAUP* 4.0b10 with maximum-likelihood analysis, a heuristic search with the starting tree obtained by neighbour-joining and tree bisection–reconnection. Gaps were handled as missing data. Due to time and computer constraints, bootstrap values were obtained with parsimony analysis. Bootstrap values were based on 1000 replications; values <50 % were not recorded. Complete D1/D2 and ITS region sequences are available in GenBank (Scorzetti *et al.*, 2002). ITS region phylogenetic data are recorded in Fig. 1 and the D1/D2 analysis is available as supplementary material in IJSEM Online.

RESULTS AND DISCUSSION

Latin diagnosis of *Trichosporon vadense* sp. nov. Middelhoven, Scorzetti *et* Fell

In medio liquido dextrosum et peptonum et extractum levidinis et extractum malti continente post 3 *dies ad* 25 °C *hyphae septatae ramosae et pseudomycelium fragmentans formantur, neque cellulae zymosae gemmantes. Sedimentum et pellicula tenuis formantur, quae etiam post* 4 *hebdomades adsunt. Cultura in agaro* PDA *dicto post* 3 *dies cremea, butyrosa, non nitida, non elevata, mycelio fimbriata; post hebdomades* 4, 20 °C, *eadem forma. In agaro* YMA *dicto post dies* 3, 20 °C, *cellulae ovoideae et hyphae fragmentantes formantur. Fermentatio nulla.* D-*Glucosum,* D-*galactosum, acetyl-*D-*glucosaminum,* D-*ribosum,* D-*xylosum,* L-*arabinosum* (*lente*), L-*rhamnosum* (*lente*), *sucrosum, maltosum, trehalosum, methyl-*α-D-*glucosidum, cellobiosum, salicinum* (*lente*), *arbutinum, melibiosum, lactosum, raffinosum* (*lente*), *glycerolum, ribitolum* (*lente*), *xylitolum* (*lente*), D-*glucitolum,* D-*mannitolum, inositolum, acidum gluconicum, acidum* 2-*ketogluconicum, acidum* 5-*ketogluconicum, acidum glucuronicum, acidum lacticum, acidum succinicum, acidum citricum* (*lente*), *ethanolum, propano*-1,2-*diolum, acidum galactonicum assimilantur, neque* L-*sorbosum,* D-*glucosaminum, melezitosum, inulinum, amylum solubile, erythritolum,* L-*arabinitolum, galactitolum, gluconolactonum, acidum galacturonicum, methanolum, butano*-2,3-*diolum, acidum quinicum. Aethylaminum,* L-*lysinum, cadaverinum et glucosaminum* (*lente*) *assimilantur, neque kalii nitratum, natrii nitritum, creatinum, creatininum, imidazolum et* D-*tryptophanum. Thiaminum externum crescentiae necessarium. Reactio Diazonii coerulaei* B *positiva.* 30 °C *crescit neque* 33 °C. *Ureum finditur. Materia amyloidea formatur* (*lente*).

Typus CBS 8901^T *isolatus ex terra prope* Wageningen *in Neerlandia, lyophilus praeservatus in collectione zymotica* Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands.

Downloaded from www.microbiologyresearch.org by
IP: 93.91.26.97
On: Fri, 27 Nov 2015 09:58:33

Table 1. Strains studied

Species	Strain	Source
T. aquatile Hedrick *et* Dupont	CBS 5973^T	Water
T. asahii Akagi *ex* Sugita, Nishikawa *et* Shinoda	CBS 2479^T	Nail of patient with psoriasis
	CBS 8520	Rotting *Euphorbia canariensis*
	M252	Snail excrements
	CBS 8904	Maize cobs
T. asteroides Rischin (Ota)	CBS 2481^T	Human skin
T. brassicae Nakase	CBS 6382^T	Cabbage
T. caseorum (sp. nov., to be described by other authors)	CBS 9052^T	Cheese
T. coremiiforme (M. Moore) Guého *et* M. Th. Smith	CBS 2482^T	Head lesion
	CBS 7137	Parrot droppings
	HB 480	Cheese
	HB 481	Cheese
T. cutaneum (de Beurmann, Gougerot *et* Vaucher) Ota	CBS 2466^T	Skin lesion
T. debeurmannianum Sugita, Takashima, Nakase, Ichikawa, Ikeda *et* Shinoda	CBS 1896^T	Bronchial secretion
T. dehoogii sp. nov.	CBS 8686^T	Unknown origin, probably from soil
T. dermatis Sugita, Takashima, Nakase, Ichikawa, Ikeda *et* Shinoda	CBS 2043^T	Infected skin
	CBS 8381	Sea water near a coral reef
T. domesticum Sugita, Nishikawa *et* Shinoda	CBS 8280^T	Rotting wood
	CBS 8111	Soil
	Ham 752	Soil
T. dulcitum (Berkhout) Weijman	CBS 8257^T	Soil
	CBS 8905	Soil
T. faecale (Batista *et* Silveira) Guého *et* M. Th. Smith	CBS 4828^T	Human faeces
T. gamsii sp. nov.	CBS 8245^T	Soil
T. gracile (Weigmann *et* Wolff) Guého *et* M. Th. Smith	CBS 8189^T	Sour milk
	CBS 8518	Ensiled brewer's grains
	CBS 8519	Ensiled leek
T. guehoae Middelhoven, Scorzetti *et* Fell	CBS 8521^T	Soil
T. inkin (Oho *ex* Ota) do Carmo-Sousa *et* van Uden	CBS 5585^T	Tinea cruris
T. japonicum Sugita *et* Nakase	CBS 8641^T	Atmosphere
T. jirovecii Frágner	CBS 6864^T	Human nail
	CBS 8826	Nest of leaf-cutting ant
T. lactis (sp. nov., to be described by other authors)	CBS 9051^T	Cheese
T. laibachii (Windisch) Guého *et* M. Th. Smith	CBS 5790^T	Soil
	CBS 8900	Water
	CBS 8523	Soil
T. loubieri Morenz (Weijman)	CBS 7065^T	Cow mastitis
	CBS 8265	Soil
T. moniliiforme (Weigmann *et* Wolff) Guého *et* M. Th. Smith	CBS 2467^T	Butter
	G41	Soil
	CBS 8110	Soil
	ATCC 46490	Soil
T. montevideense (Aciole de Queiroz) Guého *et* M. Th. Smith	CBS 6721^T	Water
T. mucoides Guého *et* M. Th. Smith	CBS 7625^T	Patient with meningitis
	Ham 801	Soil
	Ham 822	Soil
T. multisporum Cochet	CBS 2495^T	Rat droppings
	CBS 9202	Decaying mushroom
T. ovoides Behrend	CBS 7556^T	Hair white piedra
T. porosum (Stautz) Middelhoven, Scorzetti *et* Fell	CBS 2040^T	Exudate of *Taxus baccata*
	CBS 8396	Soil
	CBS 8522	Soil
T. pullulans (Lindner) Diddens *et* Lodder	CBS 2532^T	Air
T. pullulans (Lindner) Diddens *et* Lodder	CBS 9201	Leaves of *Sequoia sempervirens*

Downloaded from www.microbiologyresearch.org by
IP: 93.91.26.97
On: Fri, 27 Nov 2015 09:58:33

Table 1. cont.

Species	Strain	Source
T. scarabaeorum sp. nov.	CBS 5601^T	Scarab beetle
T. smithiae sp. nov.	CBS 8370^T	Soil
	CBS 9249	Soil
T. sporotrichoides (van Oorschot) van Oorschot *et* de Hoog	CBS 8246^T	Soil
T. vadense sp. nov.	CBS 8901^T	Soil
T. veenhuisii Middelhoven, Scorzetti *et* Fell	CBS 7136^T	Buffalo dung
Hyalodendron lignicola Diddens var. *undulatum* Diddens	CBS 220·34^T	Wood pulp
Hyalodendron lignicola Diddens var. *simplex* Diddens	CBS 221·34^T	Wood pulp
Novel species to be described elsewhere	CBS 8903	Soil

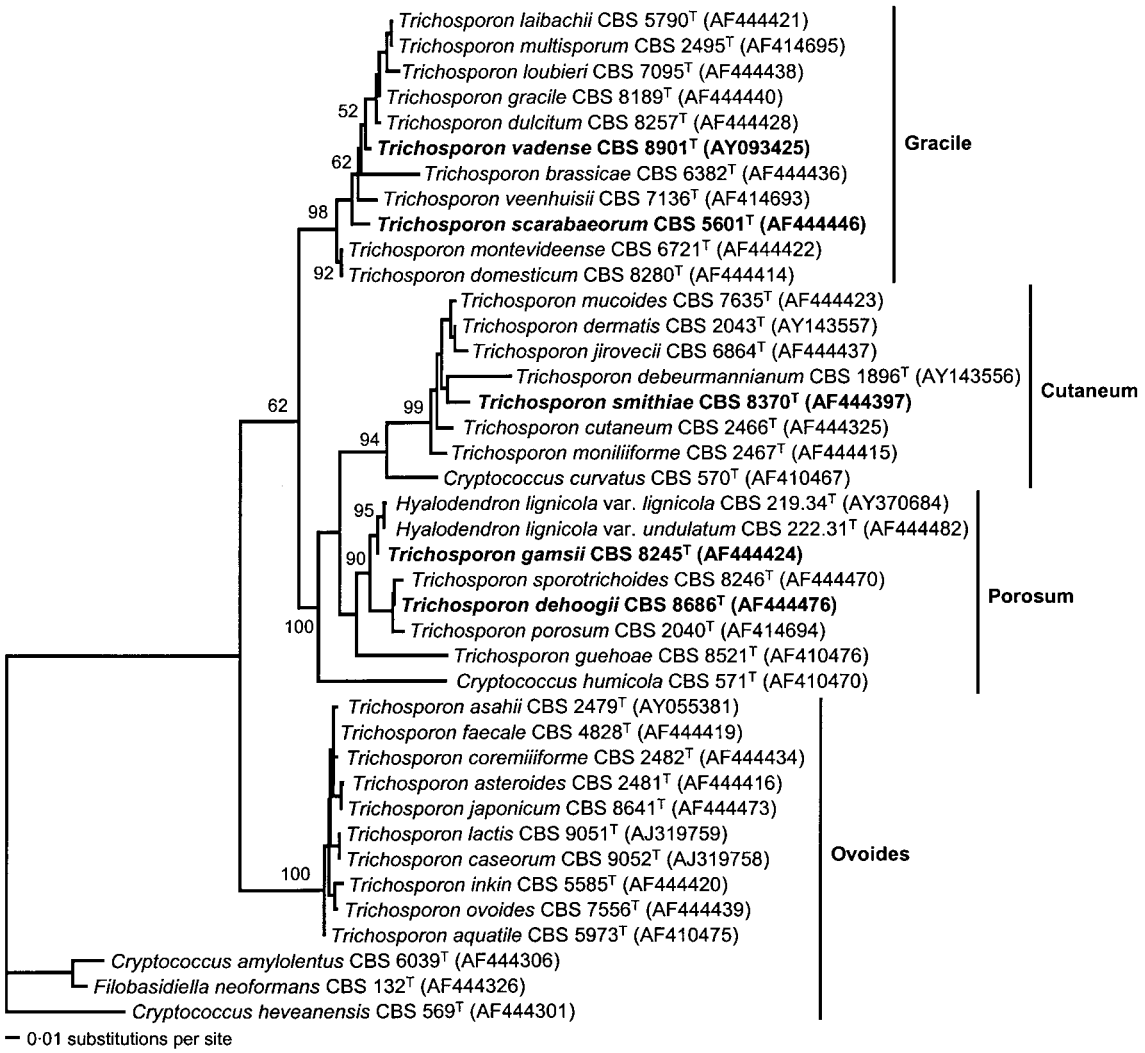

Fig. 1. Phylogenetic tree of the genus *Trichosporon* Behrend, based on nuclear sequencing of the ITS region of rDNA. Phylogenetic analysis (PAUP 4.0b10) was done by using maximum-likelihood and a heuristic search. Numbers on branches are bootstrap percentages (>50 %) from 1000 full heuristic replications, based on parsimony analysis. *Cryptococcus amylolentus*, *Filobasidiella neoformans* and *Cryptococcus heveanensis* were used as the outgroup.

Downloaded from www.microbiologyresearch.org by
IP: 93.91.26.97
On: Fri, 27 Nov 2015 09:58:33

Description of *Trichosporon vadense* sp. nov.

Trichosporon vadense (va.den′se. L. neut. adj. *vadense* referring to the Latin name of Wageningen, *Vila Vada*; during the Roman age, *Vila Vada* was a small settlement and garrison on the northern bank of the river Rhine at the northern limit of the Roman empire on the European continent).

After 3 days at 25 °C in liquid growth medium that contains glucose (1 %, w/v), yeast extract (0·3 %, w/v), peptone (0·5 %, w/v) and malt extract (0·3 % w/v), abundant branched septate mycelium and fragmenting pseudomycelium, but no budding yeast cells, are formed. A sediment and a thin pellicle are formed, which remain present after 4 weeks. Slant cultures on YM agar after 3 days at 25 °C have a butyrous texture and are cream-coloured and dull in appearance with entire to lobed margins; their appearance does not change over 4 weeks. In slide cultures on yeast morphology agar (YMA) after 3 days at 20 °C, masses of ovate cells budding on a broad base are formed. Under the cover slip, the mycelium splits into long fragments (five times as long as wide). Chains of ovate cells, budding on a broad base, are formed terminally on hyphae. Growth responses of strain CBS 8901^T on standard carbon and nitrogen compounds, as well as some other characteristics, are shown in Table 2. The predominant ubiquinone is CoQ9.

The type strain (CBS 8901^T = JCM 12194^T) was isolated in 1990 by W. J. Middelhoven from soil in Wageningen, the Netherlands, by enrichment culture on phenylacetic acid at 10 °C.

Latin diagnosis of *Trichosporon smithiae* sp. nov. Middelhoven, Scorzetti, Sugita *et* Fell

In medio liquido dextrosum et peptonum et extractum levidinis et extractum malti continente post 3 *dies* 25 °C *hyphae septatae ramosae formantur, cellulis globoseis ad ovoideis* (5–6·5 × 6·5–8·5 μm), *singulis vel binis. Sedimentum parcum repens, et pellicula alba, rugosa repens formantur, quae etiam post* 4 *hebdomades adsunt. Cultura in agaro* PDA *dicto post* 3 *dies cremea, butyrosa, non nitida, elevata et mycelio fimbriata; post hebdomades* 4, 20 °C, *eadem forma. In agaro* YMA *dicto post dies* 3, 20 °C, *mycelium, pseudomycelium et arthroconidia formantur. Fermentatio nulla.* D-*Glucosum,* D-*galactosum, acetyl*-D-*glucosaminum,* D-*ribosum,* D-*xylosum,* L-*arabinosum,* L-*rhamnosum, sucrosum, maltosum, trehalosum, methyl*-α-D-*glucosidum, cellobiosum, arbutinum, melibiosum, lactosum, raffinosum, melezitosum, amylum solubile, glycerolum* (*lente*), *erythritolum, ribitolum* (*lente*), *xylitolum* (*lente*), L-*arabinitolum* (*lente*), D-*glucitolum* (*lente*), D-*mannitolum, inositolum, acidum gluconicum, acidum* 2-*ketogluconicum, acidum glucuronicum, acidum lacticum, acidum succinicum, acidum citricum, aethanolum, propano*-1,2-*diolum et acidum galactonicum* (*lente*) *assimilantur.* L-*Sorbosum,* D-*glucosaminum,* D-*arabinosum, salicinum, inulinum, galactitolum, gluconolactonum, acidum galacturonicum, methanolum, butano*-2,3-*diolum et acidum quinicum non assimilantur. Aethylaminum,* L-*lysinum, cadaverinum et glucosaminum* (*lente*) *assimilantur, neque kalii nitratum, natrii nitritum, creatinum, creatininum, imidazolum et* D-*tryptophanum. Thiaminum externum crescentiae necessarium. Reactio Diazonii coerulaei* B *positiva.* 30 °C *crescit neque* 32 °C. *Ureum finditur. Materia amyloidea non formatur.*

Typus CBS 8370^T *isolatus ex terra prope* Angra dos Reis *in Brasilio, lyophilus praeservatus in collectione zymotica* Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands.

Description of *Trichosporon smithiae* sp. nov.

Trichosporon smithiae [smi′thi.ae. N.L. gen. n. *smithiae* in honour of Maudy Th. Smith of CBS Delft/Utrecht, specialist in ascomycetous yeast systematics and co-author of revisions of the genus *Trichosporon* (Guého *et al.*, 1992, 1998)].

After 3 days at 25 °C in liquid growth medium that contains glucose (1 %, w/v), yeast extract (0·3 %, w/v), peptone (0·5 %, w/v) and malt extract (0·3 % w/v), septate branched mycelium, budding pseudomycelium and budding globose to ovate cells (5–6·5 × 6·5–8·5 μm) are present. A flocculent sediment and a thick, wrinkled, white creeping pellicle are formed, which are present after 4 weeks. Slant cultures on PDA agar after 3 days at 25 °C have a butyrous texture and are wrinkled, cream-coloured and dull in appearance with lobed margins. After 4 weeks, cultures are yellowish. In slide cultures on YMA after 3 days at 25 °C, abundant true mycelium with arthroconidia is formed. Growth responses of strain CBS 8370^T on standard carbon and nitrogen compounds and other characteristics are shown in Table 2.

The type strain (CBS 8370^T = DBVPG 4506^T = JCM 12195^T) was isolated in 1995 by Guido Capriotti from soil in Angra dos Reis, Brazil.

During the preparation of this manuscript, Dr T. Sugita brought a strain (M-9955) to our attention that he had isolated from soil in Japan. Its D1/D2 nucleotide sequence (GenBank accession no. AB086381) is identical to the sequence of CBS 8370^T. Strain M-9955 has been deposited in the CBS yeast culture collection (accession no. CBS 9249). Physiologically, CBS 9249 differs from the type strain by failure to grow on ribitol, xylitol and L-arabinitol, compounds that support weak and slow growth of CBS 8370^T.

Latin diagnosis of *Trichosporon dehoogii* sp. nov. Middelhoven, Scorzetti *et* Fell

In medio liquido dextrosum et peptonum et extractum levidinis et extractum malti continente post 3 *dies* 25 °C *cellulae globosae ad ovoideae* (5–6·5 × 6·5–7·5 μm), *singulae vel binae, parcum pseudomycelium* (6·5 × 30 μm) *formatur. Sedimentum densum album et pellicula tenuis repens formantur, quae etiam post* 4 *hebdomades adsunt. Cultura in agaro* PDA *dicto post* 3 *dies albida, butyrosa, non nitida, non elevata, mycelio fimbriata; post hebdomades* 4, 20 °C,

Downloaded from www.microbiologyresearch.org by
IP: 93.91.26.97
On: Fri, 27 Nov 2015 09:58:33

Table 2. Physiological characteristics of the five novel *Trichosporon* species

Strains: 1, *T. vadense* CBS 8901^T; 2, *T. smithiae* CBS 8370^T; 3, *T. dehoogii* CBS 8686^T; 4, *T. scarabaeorum* CBS 5601^T; 5, *T. gamsii* CBS 8245^T. All five type strains grew on D-glucose, D-ribose, D-xylose, maltose, α-trehalose, cellobiose, lactose, 2-ketogluconate, D-gluconate, D-glucuronate, DL-lactate, ethanol, Tween 80 and acetyl-D-glucosamine as sole carbon sources and on L-lysine and cadaverine as sole nitrogen sources. All five strains failed to grow on inulin, methanol, butane-2,3-diol, *meso*-tartrate and ethylene glycol as carbon sources and on nitrate, imidazole and D-tryptophan as nitrogen sources. All five strains required thiamin as the sole external vitamin for growth and showed positive responses to the urease reaction and the diazonium blue B stain. None of the strains grew in 10 % NaCl or 5 % D-glucose or at pH 9·5. +, Growth within 8 days; −, no growth after 20 days; D, growth after 8 days or more; W, weak growth response; Th, thiamin required.

Characteristic	1	2	3	4	5
Carbon compounds:					
D-Galactose	+	+	+	W	+
L-Sorbose	−	−	D	D	+
D-Glucosamine	−	−	D	D	DW
D-Arabinose	W	−	−	−	−
L-Rhamnose	D	+	+	−	+
Sucrose	+	+	+	DW	+
Methyl α-glucoside	+	+	D	−	+
Salicin	D	−	D	−	D
Arbutin	+	+	+	−	+
Melibiose	+	+	+	−	+
Raffinose	D	+	+	−	−
Melezitose	−	+	+	−	+
Soluble starch	−	+	+	D	+
Glycerol	+	D	D	+	D
meso-Erythritol	−	+	+	−	−
Ribitol	D	D	−	+	−
Xylitol	D	D	−	DW	D
L-Arabinitol	−	D	−	−	−
D-Glucitol	+	D	D	D	+
D-Mannitol	+	+	D	+	D
Galactitol	−	−	+	−	−
myo-Inositol	+	+	+	−	+
Glucono-δ-lactone	−	−	+	−	+
5-Ketogluconate	+	D	+	+	+
Galacturonic acid	−	−	+	−	+
Succinate	+	+	+	D	+
Citrate	D	+	+	−	+
Propane-1,2-diol	+	+	D	+	+
Quinic acid	−	−	+	−	+
Hemiglucarate	+	−	+	−	+
Galactonate	+	D	+	+	+
Palatinose	+	+	+	−	+
Levulinate	+	−	D	−	−
L-Malate	+	D	+	DW	+
L-Tartrate	−	−	+	−	−
D-Tartrate	−	−	+	−	−
Galactarate	D	−	+	−	+
Gentobiose	+	D	+	−	+
Tween 60	+	D	+	+	+
Nitrogen compounds:					
Nitrite	−	−	−	−	+
Ethylamine (N)	+	+	+	D	+

Downloaded from www.microbiologyresearch.org by
IP: 93.91.26.97
On: Fri, 27 Nov 2015 09:58:33

Table 2. cont.

Characteristic	1	2	3	4	5
Creatine	−	−	+	−	D
Creatinine	−	−	+	−	+
D-Glucosamine (N)	D	DW	DW	−	−
D-Proline	+	+	+	+	D
Putrescine	D	−	+	−	+
Urotropine	+	DW	+	DW	+
Miscellaneous:					
Cycloheximide (0·01 %)	+	+	+	+	−
Cycloheximide (0·1 %)	D	D	−	D	−
Growth at pH 3·0	DW	DW	+	−	+
Amyloid production	D	−	+	−	D
Maximum growth temp. (°C)	30+, 33−	30+, 32−	30+, 32−	33+, 35−	27+, 29−

eadem forma. In agaro extracto malti confecto post dies 3, 20 °C, *hyphae septatae fragmentantes, pseudomycelium et blastoconidia formantur. Fermentatio nulla.* D-*Glucosum,* D-*galactosum,* L-*sorbosum* (*lente*), D-*glucosaminum* (*lente*), *acetyl*-D-*glucosaminum,* D-*ribosum,* D-*xylosum,* L-*arabinosum,* L-*rhamnosum, sucrosum, maltosum, trehalosum, methyl*-α-D-*glucosidum* (*lente*), *cellobiosum, salicinum* (*lente*), *arbutinum, melibiosum, lactosum, raffinosum, melezitosum, amylum solubile, glycerolum* (*lente*), *erythritolum, ribitolum* (*lente*), D-*glucitolum* (*lente*), *mannitolum* (*lente*), *galactitolum, inositolum, gluconolactonum, acidum gluconicum, acidum* 2-*ketogluconicum, acidum* 5-*ketogluconicum, acidum glucuronicum, acidum galacturonicum, acidum lacticum, acidum succinicum, acidum citricum, aethanolum, propano*-1,2-*diolum* (*lente*), *acidum quinicum et acidum galactonicum assimilantur.* D-*Arabinosum, inulinum, ribitolum, xylitolum,* L-*arabinitolum, methanolum, butano*-2,3-*diolum non assimilantur. Aethylaminum,* L-*lysinum, cadaverinum, creatinum, creatininum et glucosaminum* (*lente*) *assimilantur, neque kalii nitratum, natrii nitritum, imidazolum,* D-*tryptophanum. Thiaminum externum crescentiae necessarium. Reactio Diazonii coerulaei* B *positiva.* 30 °C *crescit neque* 32 °C. *Ureum finditur. Materia amyloidea formatur.*

Typus CBS 8686^T, *origine ignota, lyophilus praeservatus in collectione zymotica* Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands.

Description of *Trichosporon dehoogii* sp. nov.

Trichosporon dehoogii [de.hoo′gi.i. N.L. gen. n. *dehoogii* in honour of G. S. de Hoog of CBS Baarn/Utrecht, specialist in systematics of yeast-like fungi and co-author of revisions of the genus *Trichosporon* (Guého *et al.*, 1992, 1998)].

After 3 days at 25 °C in liquid growth medium that contains glucose (1 %, w/v), yeast extract (0·3 %, w/v), peptone (0·5 %, w/v) and malt extract (0·3 %, w/v), ovate budding cells and globose single cells(5–6·5 × 6·5–7·5 μm) are present. Some pseudomycelium (6·5 × 30 μm) is present. A dense white sediment and a thin creeping pellicle are formed, which remain present after 4 weeks. Slant cultures on PDA agar after 3 days at 25 °C have a butyrous texture and are flat, white to cream-coloured, have a dull appearance and a fringe of mycelium; their appearance does not change over 4 weeks. In slide cultures on malt extract agar after 3 days at 25 °C, abundant, true septate, long, fragmented and branched mycelium with globose blastoconidia is formed. Growth responses of strain CBS 8686^T on standard carbon and nitrogen compounds and other characteristics are shown in Table 2. The predominant ubiquinone is CoQ9.

The origin of the type strain of *T. dehoogii* (CBS 8686^T = JCM 12196^T) is unknown.

Latin diagnosis of *Trichosporon scarabaeorum* sp. nov. Middelhoven, Scorzetti *et* Fell

In medio liquido dextrosum et peptonum et extractum levidinis et extractum malti continente post 3 *dies* 25 °C *cellulae ovoideae ad cylindricae* (5–6·5 × 8·5–12·5 μm), *singulae vel binae. Sedimentum albidum et pellicula formantur, quae etiam post* 4 *hebdomades adsunt. Cultura in agaro* PDA *dicto post* 3 *dies albida, butyrosa, non nitida, non elevata, post hebdomades* 4, 20 °C, *cremea, eadem forma. In agaro extracto malti confecto post dies* 3, 20 °C, *mycelium, pseudomycelium et arthroconidia formantur. Fermentatio nulla.* D-*Glucosum,* L-*sorbosum* (*lente*), D-*glucosaminum* (*lente*), *acetyl*-D-*glucosaminum,* D-*ribosum,* D-*xylosum, maltosum, trehalosum, cellobiosum, lactosum, amylum solubile* (*lente*), *glycerolum, ribitolum,* D-*glucitolum* (*lente*), D-*mannitolum, acidum gluconicum, acidum* 2-*ketogluconicum, acidum* 5-*ketogluconicum, acidum glucuronicum, acidum lacticum, acidum succinicum* (*lente*), *aethanolum, propano*-1,2-*diolum et acidum galactonicum assimilantur.* L-*Arabinosum,* D-*arabinosum,* L-*rhamnosum, methyl*-α-D-*glucosidum, salicinum, arbutinum, melibiosum, raffinosum, melezitosum, inulinum, erythritolum,* L-*arabinitolum, galactitolum, inositolum, gluconolactonum, acidum galacturonicum, acidum citricum, methanolum, butano*-2,3-*diolum, acidum quinicum non assimilantur. Aethylaminum* (*lente*), L-*lysinum, cadaverinum assimilantur, neque kalii nitratum,*

Downloaded from www.microbiologyresearch.org by
IP: 93.91.26.97
On: Fri, 27 Nov 2015 09:58:33

natrii nitritum, *creatinum*, *creatininum*, *glucosaminum*, *imidazolum*, D-*tryptophanum*. *Thiaminum externum crescentiae necessarium*. *Reactio Diazonii coerulaei* B *positiva*. 33 °C *crescit neque* 35 °C. *Ureum finditur*. *Materia amyloidea non formatur*.

Typus CBS 5601^T *isolatus ex Coleopteris scarabaeideis*, *lyophilus praeservatus in collectione zymotica* Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands.

Description of *Trichosporon scarabaeorum* sp. nov.

Trichosporon scarabaeorum (sca.ra.bae.o′rum. N.L. gen. n. *scarabaeorum* derived from *Scarabaeum*, the Latin name of the scarabaeid beetle, from which the type strain was isolated).

After 3 days at 25 °C in liquid growth medium that contains glucose (1 %, w/v), yeast extract (0·3 %, w/v), peptone (0·5 %, w/v) and malt extract (0·3 % w/v), cells are 5–6·5 × 8·5–12·5 μm and occur singly or in pairs. Budding is on a broad base. Some branched mycelium is also formed. White sediment and a thin pellicle are formed. After 4 weeks, a white sediment and a thin, dry, dull, creeping pellicle are present. Slant cultures on PDA agar after 3 days at 25 °C have a butyrous texture, are flat, white and dull in appearance and their margins are entire; their appearance does not change over 4 weeks. In slide cultures on malt extract agar after 3 days at 25 °C, abundant ovate cells and mycelium with arthroconidia are formed. Growth responses of strain CBS 5601^T on standard carbon and nitrogen compounds and other characteristics are shown in Table 2. Strain CBS 5601^T is notable for its inability to assimilate inositol. The predominant ubiquinone is CoQ9.

The type strain (CBS 5601^T = JCM 12198^T) was isolated by C. E. Malan in South Africa from the gut of larva of a scarabaeid beetle (Coleoptera, Scarabaeidae).

Latin diagnosis of *Trichosporon gamsii* sp. nov. Middelhoven, Scorzetti, Sigler *et* Fell

In medio liquido dextrosum et peptonum et extractum levidinis et extractum malti continente post 3 *dies* 20 °C *hyphae ramosae*, *septatae formantur*, *et cellulae globosae ad ovoideae* (5–7·5 × 6·5–15 μm), *singulae vel binae*. *Sedimentum copiosum album et pellicula repens formantur*, *quae etiam post* 4 *hebdomades adsunt*. *Cultura in agaro* PDA *dicto post* 3 *dies cremea*, *butyrosa*, *non nitida*, *elevata*, *mycelio fimbriata*; *post hebdomades* 4, 20 °C, *eadem forma*. *In agaro* YMA *dicto post dies* 3, 20 °C, *hyphae septatae fragmentantes et blastoconidia formantur*. *Fermentatio nulla*. D-*Glucosum*, D-*galactosum*, L-*sorbosum*, D-*glucosaminum* (*lente*), *acetyl*-D-*glucosaminum*, D-*ribosum*, D-*xylosum*, L-*arabinosum*, L-*rhamnosum*, *sucrosum*, *maltosum*, *trehalosum*, *methyl*-α-D-*glucosidum*, *cellobiosum*, *salicinum* (*lente*), *arbutinum*, *melibiosum*, *lactosum*, *melezitosum*, *amylum solubile*, *glycerolum* (*lente*), *xylitolum* (*lente*), D-*glucitolum*, D-*mannitolum* (*lente*), *inositolum*, *gluconolactonum*, *acidum gluconicum*, *acidum* 2-*ketogluconicum*, *acidum* 5-*ketogluconicum*, *acidum glucuronicum*, *acidum galacturonicum*, *acidum lacticum*, *acidum succinicum*, *acidum citricum*, *aethanolum*, *propano*-1,2-*diolum*, *acidum quinicum assimilantur*. D-*Arabinosum*, *raffinosum*, *inulinum*, *erythritolum*, *ribitolum*, L-*arabinitolum*, *galactitolum*, *methanolum*, *butano*-2,3-*diolum non assimilantur*. *Natrii nitritum*, *aethylaminum*, L-*lysinum*, *cadaverinum*, *creatinum* (*lente*), *creatininum assimilantur*. *Kalii nitratum*, *glucosaminum*, *imidazolum*, D-*tryptophanum non assimilantur*. *Thiaminum externum crescentiae necessarium*. *Reactio Diazonii coerulaei* B *positiva*. 27 °C *crescit neque* 29 °C. *Ureum finditur*. *Materia amyloidea formatur* (*lente*).

Typus CBS 8245^T *isolatus a* W. Gams *ex terra humosa madida radicibus interspersis*, Sierra Nevada de Santa Maria *in Colombia*, *lyophilus praeservatus in collectione zymotica* Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands.

Description of *Trichosporon gamsii* sp. nov.

Trichosporon gamsii (gam′si.i. N.L. gen. n. *gamsii* in honour of W. Gams of CBS Baarn/Utrecht, specialist in fungal systematics and botanical Latin, who isolated the strain).

After 3 days at 25 °C in liquid growth medium that contains glucose (1 %, w/v), yeast extract (0·3 %, w/v), peptone (0·5 %, w/v) and malt extract (0·3 % w/v), cells are 5–7·5 × 6·5–15 μm in size. A thick, white sediment and a slightly creeping thin pellicle are formed. After 4 weeks, a heavy white sediment and a dry, creeping pellicle are present. Slant cultures on PDA agar after 3 days at 25 °C are membranous, wrinkled, cream-coloured and dull in appearance with lobed margins; their appearance does not change over 4 weeks. In slide cultures on YMA after 3 days at 25 °C, abundant, branched and septate mycelium with ovate blastoconidia are formed. Under the cover slip, budding ovate cells and long, fragmented hyphae (ten times as long as wide) are formed. Growth responses of strain CBS 8245^T on standard carbon and nitrogen compounds and other characteristics are shown in Table 2. The predominant ubiquinone is CoQ9.

The type strain (CBS 8245^T = JCM 12197^T) was isolated by W. Gams from a sample of moist humus around roots that was collected by T. van der Hammen in the Sierra Nevada de Santa Maria, Colombia. In previous papers (Guého *et al.*, 1998; Middelhoven *et al.*, 2001), CBS 8245 was erroneously considered to be the type strain of *Trichosporon sporotrichoides*.

Non-conventional carbon sources

Table 3 records growth responses to carbon compounds that are not traditionally used in yeast taxonomics. The pattern of carbon-compound assimilation was species-specific for the majority of species (Table 3). An identification key for clinically relevant *Trichosporon* species, based on some of these characteristics, was published recently (Middelhoven, 2003). A dichotomous identification key

Downloaded from www.microbiologyresearch.org by
IP: 93.91.26.97
On: Fri, 27 Nov 2015 09:58:33

Table 3. Diagnostic growth responses of 36 species of the genus *Trichosporon* Behrend

Characters: 1, uric acid; 2, polygalacturonate; 3, ethylamine; 4, L-4-hydroxyproline; 5, quinate pH 5·5; 6, orcinol; 7, phloroglucinol; 8, 4-ethylphenol; 9, 2,3-dihydroxybenzoate; 10, tyramine; 11, L-phenylalanine; 12, erythritol; 13, L-rhamnose; 14, melibiose; 15, raffinose; 16, melezitose; 17, galacturonic acid; 18, maximum growth temperature (°C).

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
T. pullulans	−	+	−	−	−	−	−	−	−	−	−	+	+D	+	+	+D	+D	23+, 27−
Gracile clade:																		
T. laibachii	+	+	−D	V	V	+	−	+	+	+	+D	−	+	+	+	V	V	28+, 32−
T. multisporum	+	+	−	V	−	+	−	−	+	V	+D	−	V	+	+	+	−	30+, 35−
T. loubieri	−	V	−	+D	+	+D	−	−	+D	+D	−	−	+D	+	+	−D	V	42+, 45−
T. gracile	−	−	−	−D	−D	+	−	V	DW	−	+	−	−	−	−	−	−	30+, 35−
T. dulcitum	−	DW	−	+D	−	−	−	V	V	−	+	−W	−D	−	V	V	−	25+, 30−
T. vadense	V	+	D	+	+D	−	−	−	+	+	D	−	+	+	D	−	−	30+, 33−
T. brassicae	−	−	−	+	−	−	−	−	+	+	+	−	−	−	−	−	D	37+, 40−
T. veenhuisii	+	−	−	−	D	−	−	−	+	D	−	−	−	−	D	DW	−	43+, 45−
T. scarabaeorum	−	−	−	+	−	−	−	+	+	−	−	−	−	−	−	−D	−	32+, 35−
T. montevideense	+	−	+	+	+	−	−	−	D	−	+	V	V	−	−	V	−	35+, 40−
T. domesticum	+	−	+	+	−D	−	−	−	D	+	+	−	−	−	−	+	−	35+, 40−
Cutaneum clade:																		
T. mucoides	−	+	+	V	V	+	D	−	+D	+D	V	+	+	+	+	+D	V	37+, 42−
T. dermatis	−	+	−	+	+	+	−	−	+	−	+	+	+	V	+	+	+	37+, 40−
T. jirovecii	+	+	+	+	V	+	−	−	+	+D	+	+	+	+	+	+	−	30+, 35−
T. debeurmannianum	+	−	+	+	−	−	−	−	+	+	D	+	+	+	+	DW	DW	35+, 37−
T. smithiae	+	+	+	+	+	−	−	−	+	+	D	+	+	+	+	+	−	30+, 32−
T. cutaneum	−	−	−	+	+	D	−	−	D	−	+	V	+D	+D	V	+	−	33+, 37−
T. moniliiforme	V	+D	V	V	+	V	+D	V	+	V	+D	+	+	+	+	+D	V	28+, 32−
Porosum clade:																		
H. lignicola	+	W	V	−	+	−	−	−	−	+D	+	+	+	+	+	+	?	28+, 30−
T. gamsii	+	+	−	−	+	D	D	−	−	−	+D	−	+	+	−	+	+	27+, 29−
T. wieringae	+	+	−	+	+	+	+	−	−	−	+D	−	+	+	+	+	D	34+, 35−
T. sporotrichoides	−	+	−	−D	+	+	+	−	+	+	−D	−	+	+	+	+	+	30+, 35−
T. dehoogii	−	D	D	D	+	+	DW	+	+	D	+	+	+	+	+	+	+	30+, 32−
T. porosum	−	+	−D	V	+	+	+D	V	+D	+	V	+	+	+	+	+	+	28+, 31−
T. guehoae	−	D	+	+	+	−	+	−	+	D	+D	−	+	+	+	+	+	37+, 40−
Ovoides clade:																		
T. asahii	+	−	V	−D	−	−	−	V	−D	V	+	V	+D	−D	−D	V	−	37+, 42−
T. faecale	+	−	+	+	−	+	−	−	D	−	+	+	+	−	−	D	−	40+, 42−
T. coremiiforme	+	−	+	+D	−	+D	−	V	V	+	+	+	+	−	−	+	−	40+, 42−
T. asteroides	+	−	+	−	−	−	−	−	−	−	+	V	V	−	−	+D	−	37+, 42−
T. japonicum	+	−	D	−	−	D	−	−	−	+	+D	+	−	−	−	+	W	39+, 40−
T. lactis	+D	−	−	−	−	−	−	−	DW	−	D	+	−	−	−	+	−	35+, 37−
T. caseorum	−	DW	−	−	−	−	−	−	−	−	+	+	−	−	−	+	−	37+, 40−
T. inkin	+	−	−	D	−	−	−	−	−	+	+	+D	−	−	−	+D	−	37+, 45−
T. ovoides	+	−	D	−	−	+D	−	+	D	+	D	V	+D	−D	V	V	−	35+, 40−
T. aquatile	+	−	+	−	−	−	−	−	−	+	+	−D	−	−	−	−D	W−	35+ 37−

to 36 *Trichosporon* species, based on these growth tests, maximum growth temperature and assimilation of erythritol and L-rhamnose, is available as supplementary material in IJSEM Online. Delayed growth responses (D) are treated as + and −, so that strains can be identified after 1 week incubation. However, incubation for another week is recommended. Comparison of the data with those presented in Table 3 is required to confirm identification. Species that cannot be distinguished by standard techniques (e.g. *Trichosporon asahii* from *Trichosporon coremiiforme* and *Trichosporon cutaneum* from *Trichosporon moniliiforme*) can be separated by the following characters shown in Table 3: growth on L-4-hydroxyproline and orcinol, polygalacturonate, phloroglucinol and tyramine. In contrast, compounds to separate *T. asahii* and *Trichosporon asteroides* have not yet been found.

The benzene compounds listed in Table 4 are assimilated

Downloaded from www.microbiologyresearch.org by
IP: 93.91.26.97
On: Fri, 27 Nov 2015 09:58:33

Table 4. Benzene compounds that are assimilated by almost all *Trichosporon* strains studied

Compounds are listed in order of the length of their side chain.

Compound
Phenol
Catechol (1,2-dihydroxybenzene)
Resorcinol (1,3-dihydroxybenzene)
Hydroquinone (1,4-dihydroxybenzene)
Benzoate
3-Hydroxybenzoate
4-Hydroxybenzoate
Protocatechuate (3,4-dihydroxybenzoate)
Phenylacetate
2-Hydroxyphenylacetate
3-Hydroxyphenylacetate
4-Hydroxyphenylacetate
Homoprotocatechuate (3,4-dihydroxyphenylacetate)
Cinnamate
3-Hydroxycinnamate
4-Hydroxycinnamate
Caffeate (3,4-dihydroxycinnamate)

as sole carbon sources by many yeast species, including species of *Trichosporon* (Middelhoven *et al.*, 1992, 1999, 2000, 2001; Middelhoven, 1993; Sampaio, 1999). Consequently, these particular compounds have no diagnostic value, in contrast to other benzene compounds, e.g. orcinol, phloroglucinol, 4-ethylphenol, 2,3-dihydroxybenzoate, tyramine and L-phenylalanine, which can be used to distinguish species of *Trichosporon* (Table 3). *T. pullulans* is an exception: the type strain does not grow on any of these benzene compounds, thus confirming its remote phylogenetic relationship to other species of the genus.

Some compounds were included specifically in this study, due to their presence in natural habitats. Uric acid, which occurs in small amounts in soil, is the nitrogenous end product of protein catabolism in birds, reptiles and insects. Several yeast species utilize uric acid as the sole source of carbon and energy (Middelhoven *et al.*, 1983, 1985, 1991). Some of these strains were associated with birds or had been isolated from soil by enrichment culture on uric acid. Growth on uric acid, which generally occurred within 5 days, proved to be a useful identification tool. All species of the genus *Trichosporon* with the exception of *T. moniliiforme* and *T. vadense* demonstrated definite positive or negative growth responses.

L-4-Hydroxyproline is a building block of collagen (which is found in cartilage, connective tissue and gelatin), from which the compound is released by enzymic hydrolysis. L-4-Hydroxyproline is assimilated by the pathogenic species *T. coremiiforme*, *T. cutaneum*, *Trichosporon dermatis*, *Trichosporon inkin*, *Trichosporon jirovecii* and *Trichosporon loubieri*, but not by *Trichosporon asahii* (some strains can assimilate it slowly), *Trichosporon asteroides* or *Trichosporon ovoides*. *Trichosporon mucoides* showed variable growth responses to this hydroxyamino acid, which was also assimilated by some species that were unable to grow at 35 °C (*Trichosporon dulcitum*, *Trichosporon multisporum*, *T. scarabaeorum*, *T. smithiae* and *T. vadense*).

Phloroglucinol and polygalacturonate are typical plant constituents. Few of the pathogenic species were able to assimilate these carbon compounds: phloroglucinol was assimilated by *T. mucoides* and polygalacturonate by *T. dermatis*, *T. jirovecii* and *T. mucoides*. *T. loubieri* was variable for this characteristic. Other typical plant constituents, such as quinic acid and orcinol, were assimilated by several pathogenic and non-pathogenic species.

Phylogenetic analysis of the genus and novel species

Sugita & Nakase (1998) reported the presence of three groups of species within the genus *Trichosporon*, based on SSU rDNA analysis of 20 taxa. Our study (Fig. 1), with an expanded number of taxa ($n=36$), concurs with the identity of these three groups, which correspond to our informal clade designations of Gracile (Sugita & Nakase group III), Cutaneum (group I) and Ovoides (group II). Scorzetti *et al.* (2002) recognized a fourth clade, Hyalodendron, presently named Porosum, which was represented in the Sugita & Nakase SSU rDNA tree as a separate branch. Several species within the Trichosporonales have not been included in our study: specifically, *Trichosporon terricola* (Sugita *et al.*, 2002) became available too late for inclusion in the present study. The GenBank accession number for the sequence of the ITS and D1/D2 regions is AB086382. There are several *Cryptococcus* species among the Trichosporonales: *Cryptococcus curvatus* (Fig. 1 and Supplementary Figure, available in IJSEM Online) and *Cryptococcus haglerorum* (Middelhoven *et al.*, 2003), which appear to be related to the Cutaneum clade, and *Cryptococcus humicola*, which may represent a separate cluster within or adjacent to the Trichosporonales (Fig. 1). Takashima *et al.* (2001) demonstrated that *Cryptococcus musae*, *Cryptococcus ramirezgomezianus*, *Cryptococcus longus* and *Cryptococcus pseudolongus*, which are phenotypically similar to *C. humicola*, represent a species complex within the Trichosporonales. The analysis by Takashima *et al.* (2001) was based on 18S rDNA; sequences for the ITS and D1/D2 regions were not available for inclusion in our study. Those authors also reported *Cryptococcus daszewskae* to be related to *C. curvatus*. Clearly, a systematic evaluation of *Cryptococcus* species within the Trichosporonales is required.

Comparison of the ITS and D1/D2 Trichosporonales trees (Fig. 1 and Supplementary Figure, available in IJSEM Online) demonstrates more variability in the D1/D2 region than in the ITS region. For example, we observed no differences in the ITS sequence alignments between *Trichosporon laibachii* and *T. multisporum*, and seven differences (transitions : transversions, 6 : 1) in the D1/D2 region.

Downloaded from www.microbiologyresearch.org by
IP: 93.91.26.97
On: Fri, 27 Nov 2015 09:58:33

Trichosporon montevideense and *Trichosporon domesticum* had identical sequences in the ITS region and differed at two positions (ti : tv, 1 : 1) in D1/D2. SSU rDNA analyses also demonstrated nucleotide position differences within these pairs of species (Sugita & Nakase, 1998). The general assumption is that LSU rDNA is less variable than the ITS region. The reason for the lack of conformity to this assumption among the Trichosporonales is not clear. Yang & Yoder (1999) examined ti : tv rate ratios and found that they varied between genes and lineages. These authors, however, could not find a biological explanation for these rate variations.

Previous studies (Middelhoven *et al.*, 1999, 2000, 2001) that focused on the D1/D2 region provided ample sequence differences between species. However, the inclusion of the ITS region suggests that there may be closer phylogenetic relationships among species than was considered previously. In some cases, this relationship is reflected in phenotypic characteristics. A prime example is the species group *T. coremiiforme*, *T. asteroides* and *T. asahii*, which are similar in phenotypic characters and the ITS region, but differ in D1/D2 sequence. Species differentiation for such closely related species may require confirmation by using other sequences, such as rDNA intergenic spacer (IGS) sequences, as demonstrated by Sugita *et al.* (2002). These authors examined 43 strains of 25 species of *Trichosporon* in the IGS region and reported geographical substructure among strains of *T. asahii* and genetic differences between *T. asahii*, *T. coremiiforme*, *Trichosporon faecale*, *T. asteroides* and *T. japonicum*.

The five novel species, which reside in the Gracile, Porosum and Cutaneum clades, can be differentiated readily by genetic and phenotypic differences. The latter are shown in Tables 2 and 3. Distinction of the four clades by these phenotypic characters is difficult, confirming the monophyletic nature of the genus.

The Gracile clade has strong support in the ITS region (bootstrap value, 99 %). This clade includes the two novel species *T. vadense* and *T. scarabaeorum*. The Gracile clade is physiologically heterogeneous with respect to maximum growth temperature and assimilation of polygalacturonate, uric acid, orcinol, L-4-hydroxyproline, tyramine and L-phenylalanine. Most species do not grow on ethylamine, but *T. domesticum* and *T. montevideense*, which take a separate position in this clade, grow well on this amine as the sole source of carbon and nitrogen (Table 3). According to the ITS-based phylogenetic tree (Fig. 1), the closest relatives of *T. vadense* are *T. laibachii*, *T. multisporum*, *T. loubieri*, *T. dulcitum*, *Trichosporon gracile* and *Trichosporon veenhuisii*, although statistical support is weak (52 %). All of these species have some characteristics in common, e.g. failure to grow on erythritol, galacturonic acid (*T. laibachii* is variable) and phloroglucinol, no or delayed growth on ethylamine as the sole source of carbon and nitrogen and a maximum growth temperature of 35 °C or less, with the exception of *T. veenhuisii* and *T. loubieri*, which grow at 42 °C. Physiologically, *T. vadense* is similar to *T. laibachii* and *T. multisporum*. These three species can be separated from each other by growth responses to melezitose, orcinol and 4-ethylphenol. *T. vadense*, *T. laibachii* and *T. multisporum* grow readily on polygalacturonate, but most strains do not grow on the monomer galacturonic acid. Growth tests on the latter are traditionally carried out without prior pH adjustment, resulting in an initial pH below 3·0 (Middelhoven, 1997). Assimilation of the polymer and failure to grow on the monomer reflect sensitivity to acid growth conditions.

T. scarabaeorum is not related closely to any species in the clade, although a possible relationship to *Trichosporon brassicae*, *T veenhuisii*, *T. montevideense* and *T. domesticum* is depicted in the ITS-based tree (Fig. 1). All of these species have a maximum growth temperature between 35 and 40 °C, but that of *T. scarabaeorum* is between 32 and 35 °C. The novel species can be distinguished in the clade by a combination of responses to 4-ethylphenol and L-phenylalanine. Distinction of *T. domesticum* and *T. montevideense* within the clade can be achieved by their differential abilities to utilize quinate and tyramine. Separation by traditional growth tests is difficult, as many growth responses are variable.

The Porosum clade, which has 90 % bootstrap support in the ITS tree, includes two novel species, *T. gamsii* and *T. dehoogii*, and the species *Hyalodendron lignicola*, *T. sporotrichoides* and *Trichosporon porosum*. The ITS tree suggests weak relationships of *Trichosporon guehoae* and *C. humicola* to these species, observations that are not supported by D1/D2 analysis (see Supplementary Figure in IJSEM Online). The Porosum clade is characterized by a low maximum growth temperature (<37 °C) and by growth on L-rhamnose, melibiose, raffinose (except *T. gamsii*), galacturonic acid and quinate. The novel species *T. gamsii* can be distinguished in the clade by the combined characters of assimilation of uric acid and failure to grow on raffinose and 2,3-dihydroxybenzoate. The other novel species in the clade, *T. dehoogii*, is similar to *T. porosum*, but does not assimilate L-arabinitol (Table 2) and utilizes creatine and creatinine as sole nitrogen sources. *T. sporotrichoides* can be distinguished from *T. porosum* and *T. dehoogii* by failure to grow on erythritol. The other member of the clade, *H. lignicola*, is characterized by very few or no budding yeast cells (de Hoog & Smith, 1998).

The Cutaneum clade (without *C. curvatus*) is supported strongly by bootstrap analysis (Fig. 1) and is characterized by coenzyme Q10 as the main ubiquinone (Sugita & Nakase, 1998) and by growth on polygalacturonate (except for *Trichosporon debeurmannianum* and *T. cutaneum*) and on 2,3-dihydroxybenzoate, erythritol, L-rhamnose and raffinose, with the exception of some strains of *T. cutaneum*. The Cutaneum clade is heterogeneous with respect to maximum growth temperature and assimilation of uric acid. The novel species *T. smithiae* does not appear to be related closely to any particular species in either tree.

Downloaded from www.microbiologyresearch.org by
IP: 93.91.26.97
On: Fri, 27 Nov 2015 09:58:33

Distinction from other species in the clade can be based on growth responses to uric acid, polygalacturonate, orcinol and phloroglucinol.

The Ovoides clade is supported strongly by bootstrap analysis (100 %; Fig. 1). All species have a maximum growth temperature of 35–40 °C, assimilate uric acid (except for *Trichosporon caseorum*) and L-phenylalanine (some strains are slow) and do not assimilate quinate or phloroglucinol. In addition, responses to melibiose, raffinose, galacturonic acid and polygalacturonate are negative for most strains.

ACKNOWLEDGEMENTS

Thanks are due to W. Gams, Utrecht, the Netherlands, for correcting the Latin diagnoses, to Elke Hammer, Greifswald, Germany, for determining the predominant coenzyme Q and for strains *T. domesticum* Ham 752 and *T. mucoides* Ham 801 and 822, to H.-J. Prillinger, Vienna, Austria, for strains *T. coremiiforme* HB 480 and HB 481 and for the type strains of *T. caseorum* and *T. lactis* prior to their publication, to Ann Vaughan-Martini for strain CBS 8370, to T. Sugita for strain CBS 9249 and to Lynne Sigler for strains and discussions regarding the phylogenetic placement of *T. sporotrichoides* and *T. gamsii*. Nucleotide sequencing and sequence analysis by J. W. F. and G. S. were supported by the USA National Science Foundation, Division of Environmental Biology (DEB 0206521).

REFERENCES

de Hoog, G. S. & Smith, M. Th. (1998). *Hyalodendron* Diddens. In *The Yeasts, a Taxonomic Study*, 4th edn, pp. 773–774. Edited by C. P. Kurtzman & J. W. Fell. Amsterdam: Elsevier.

de Hoog, G. S., Guarro, J., Gené, J. & Figueras, M. J. (editors) (2000). *Atlas of Clinical Fungi*, pp. 164–175. Utrecht: Centraalbureau voor Schimmelcultures.

Fell, J. W., Boekhout, T., Fonseca, A., Scorzetti, G. & Statzell-Tallman, A. (2000). Biodiversity and systematics of basidiomycetous yeasts as determined by large-subunit rDNA D1/D2 domain sequence analysis. *Int J Syst Evol Microbiol* **50**, 1351–1371.

Guého, E., Smith, M. Th., de Hoog, G. S., Billon-Grand, G., Christen, R. & Batenburg-van der Vegte, W. H. (1992). Contributions to a revision of the genus *Trichosporon*. *Antonie van Leeuwenhoek* **61**, 289–316.

Guého, E., Smith, M. Th. & de Hoog, G. S. (1998). *Trichosporon* Behrend. In *The Yeasts, a Taxonomic Study*, 4th edn, pp. 854–872. Edited by C. P. Kurtzman & J. W. Fell. Amsterdam: Elsevier.

Kwon-Chung, K. J. & Bennett, J. E. (1992). *Medical Mycology*. Philadelphia, PA: Lea & Febiger.

Middelhoven, W. J. (1993). Catabolism of benzene compounds by ascomycetous and basidiomycetous yeasts and yeastlike fungi. A literature review and an experimental approach. *Antonie van Leeuwenhoek* **63**, 125–144.

Middelhoven, W. J. (1997). Assimilation of organic acids: the pH as determining factor. *Yeast* **46**, 19–20.

Middelhoven, W. J. (2003). Identification of clinically relevant *Trichosporon* species. *Mycoses* **46**, 7–11.

Middelhoven, W. J. & Kurtzman, C. P. (2003). Relation between phylogeny and physiology in some ascomycetous yeasts. *Antonie van Leeuwenhoek* **83**, 69–74.

Middelhoven, W. J., van den Brink, J. A. & Veenhuis, M. (1983). Growth of *Candida famata* and *Trichosporon cutaneum* on uric acid as the sole source of carbon and energy, a hitherto unknown property of yeasts. *Antonie van Leeuwenhoek* **49**, 361–368.

Middelhoven, W. J., de Kievit, H. & Biesbroek, A. L. (1985). Yeast species utilizing uric acid, adenine, n-alkylamines or diamines as sole source of carbon and energy. *Antonie van Leeuwenhoek* **51**, 289–301.

Middelhoven, W. J., de Jong, I. M. & de Winter, M. (1991). *Arxula adeninivorans*, a yeast assimilating many nitrogenous and aromatic compounds. *Antonie van Leeuwenhoek* **59**, 129–137.

Middelhoven, W. J., Koorevaar, M. & Schuur, G. (1992). Degradation of benzene compounds by yeasts in acidic soils. *Plant Soil* **145**, 37–43.

Middelhoven, W. J., Scorzetti, G. & Fell, J. W. (1999). *Trichosporon guehoae* sp. nov., an anamorphic basidiomycetous yeast. *Can J Microbiol* **45**, 686–690.

Middelhoven, W. J., Scorzetti, G. & Fell, J. W. (2000). *Trichosporon veenhuisii* sp. nov., an alkane-assimilating anamorphic basidiomycetous yeast. *Int J Syst Evol Microbiol* **50**, 381–387.

Middelhoven, W. J., Scorzetti, G. & Fell, J. W. (2001). *Trichosporon porosum* comb. nov., an anamorphic basidiomycetous yeast inhabiting soil, related to the *loubieri/laibachii* group of species that assimilate hemicelluloses and phenolic compounds. *FEMS Yeast Res* **1**, 15–22.

Middelhoven, W. J., Fonseca, A., Carreiro, S. C., Pagnocca, F. C. & Bueno, O. C. (2003). *Cryptococcus haglerorum*, sp. nov., an anamorphic basidiomycetous yeast isolated from nests of the leaf-cutting ant *Atta sexdens*. *Antonie van Leeuwenhoek* **83**, 167–174.

Sampaio, J. P. (1999). Utilization of low molecular weight aromatic compounds by heterobasidiomycetous yeasts: taxonomic implications. *Can J Microbiol* **45**, 491–512.

Scorzetti, G., Fell, J. W., Fonseca, A. & Statzell-Tallman, A. (2002). Systematics of basidiomycetous yeasts: a comparison of large subunit D1/D2 and internal transcribed spacer rDNA regions. *FEMS Yeast Res* **2**, 495–517.

Sugita, T. & Nakase, T. (1998). Molecular phylogenetic study of the basidiomycetous anamorphic yeast genus *Trichosporon* and related taxa based on small subunit ribosomal DNA sequences. *Mycoscience* **39**, 7–13.

Sugita, T., Nishikawa, A., Ikeda, R. & Shinoda, T. (1999). Identification of medically relevant *Trichosporon* species based on sequences of internal transcribed spacer regions and construction of a database for *Trichosporon* identification. *J Clin Microbiol* **37**, 1985–1993.

Sugita, T., Takashima, M., Nakase, T., Ichikawa, T., Shinoda, T. & Nishikawa, A. (2002). A basidiomycetous anamorphic yeast, *Trichosporon terricola* sp. nov., isolated from soil. *J Gen Appl Microbiol* **48**, 293–297.

Takashima, M., Sugita, T., Shinoda, T. & Nakase, T. (2001). Reclassification of the *Cryptococcus humicola* complex. *Int J Syst Evol Microbiol* **51**, 2199–2210.

Yang, Z. & Yoder, A. D. (1999). Estimation of the transition/transversion rate bias and species sampling. *J Mol Evol* **48**, 274–283.

Yarrow, D. (1998). Methods for the isolation, maintenance and identification of yeasts. In *The Yeasts, a Taxonomic Study*, 4th edn, pp. 77–100. Edited by C. P. Kurtzman & J. W. Fell. Amsterdam: Elsevier.

Downloaded from www.microbiologyresearch.org by
IP: 93.91.26.97
On: Fri, 27 Nov 2015 09:58:33