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Polysaccharides and phenolic compounds as substrate for yeasts isolated from rotten wood and description of *Cryptococcus fagi* sp.nov.

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Abstract

Pieces of rotten wood collected in the forest were screened for the presence of yeasts. In spring time 3 tree species were sampled, followed by 9 species in summer. Yeast strains were identified by traditional methods. Identifications were confirmed by sequencing of ribosomal DNA in case of doubt. In total 14 yeast species of ascomycetous affiliation and 6 anamorphic basidiomycetous yeasts were isolated and identified. Most species were represented by only one strain, but *Candida bertae* by two and *Trichosporon porosum* by six strains, all from different wood samples. Three strains represented novel species, one of which is described as *Cryptococcus fagi* Middelhoven et Scorzetti. The type strain is CBS 9964 (JCM 13614). All strains were tested for growth on several polysaccharides as sole carbon source. Only some of these polymers supported growth of ascomycetous yeasts. Basidiomycetous yeasts assimilated soluble starch, pullulan, dextran, xylan, polygalacturonate, galactomannan and tannic acid or at least some of these. *Cryptococcus podzolicus* and *T. porosum* were the most active in this respect. None of the isolated strains grew on carboxymethyl cellulose, colloidal chitin, arabinogalactan and gum xanthan. Phenolic compounds were assimilated by several strains, belonging to the Trichosporonales and the *Microbotryum* and *Stephanoascus*/*Blastobotrys* clades, but not by members of the Tremellales (*Cryptococcus musci* excepted) and the *Debaryomyces*/*Lodderomyces* clade. Most of the ascomycetes assimilated *n*-hexadecane.

Introduction

Polysaccharide assimilation by yeasts has received little attention, but growth tests on soluble starch and inulin have been used for long for delimitation of taxa and for identification of yeast species (Kurtzman and Fell 1998; Barnett et al. 2000). Recently, assimilation of polygalacturonate and some other polysaccharides has been shown to distinguish species of the anamorphic basidiomycetous yeast genus *Trichosporon* Behrend (Middelhoven 2003, 2004; Middelhoven et al. 2004). This raised the question whether other yeast genera also show this ability. For this reason, yeast strains were isolated from habitats rich in polysaccharides, viz. decayed wood samples of several tree species, taken in the forest in two seasons, and subsequently tested for growth on several polysaccharides.

Attempts to isolate yeasts from rotten wood were made already a few decades ago by Grinbergs in 1970 and by Ramírez and González (1984a, b, and several other papers). They sampled decayed wood in the Valdivian evergreen rain forest of Southern Chile. They isolated and described in

total 18 currently accepted (Barnett et al. 2000) novel species. According to up-to-date nomenclature: 12 *Candida* spp., 1 *Pichia* sp., 1 *Schizoblastosporion* sp. and 4 *Rhodotorula* spp. This remarkable biodiversity raised our interest in a similar habitat in another part of the world, viz. a forest in Western Europe. As phenolic compounds are often present in plant material, attention was paid to the assimilation of these compounds by the isolated strains. These compounds rarely attract the attention of yeast taxonomists in spite of their abundance in plants, but growth tests on them are useful for the distinction of yeast taxa (Middelhoven 1993 and subsequent papers; Sampaio 1999).

Materials and methods

Isolation and identification of yeasts

Pure yeast cultures were obtained by streaking a suspension of the wood samples on glucose (1%), peptone (0.5%), yeast extract (0.3%), malt extract (0.3%), agar (2%), supplied with antibiotics (chloroamphenicol, streptomycin sulphate and tetracycline at 20 mg/l) in order to suppress growth of bacteria. The plates were incubated at 20 °C. Cultures of ascomycetous yeasts were maintained on slants of the same medium, basidiomycetous yeasts on potato dextrose agar ([PDA], Difco). The strains were examined for morphological and physiological properties with standard yeast identification methods (Yarrow 1998; Barnett et al. 2000). Utilization of carbon (10 g/l) and nitrogen sources (40 mM assimilable N) in liquid Difco Yeast Nitrogen Base and Yeast Carbon Base (2 ml in culture tubes of 18 mm diameter) was tested at 25 °C on a rotary shaker at a speed of 100 rpm. Utilization of nitrite was tested by the auxanographic technique. The pH of growth media was adjusted to 5.5 if required, but the pH of media with galacturonic or quinic acid was not adjusted, which is in agreement with the laboratory practice of CBS Utrecht (D. Yarrow, personal communication; cf. Middelhoven 1997). Growth on L-malic, D-galactaric (mucic, 2.5 g/l), D-glucaric (saccharic) and tartaric acids was tested at pH 4.0. Assimilation of carbon compounds other than those used in the standard species description was studied in a synthetic growth medium GB (Middelhoven et al. 1991), which differed from Difco YNB by a tenfold higher potassium phosphate concentration and by a lower concentration of ammonium chloride, 2 g/l rather than 5 g ammonium sulphate per litre. To growth media with uric acid and other nitrogenous carbon sources no ammonium chloride was added. Assimilation of *n*-hexacecane and potentially toxic phenolic compounds was studied on slants of GB agar medium (Middelhoven et al. 1991, 2004).

For identification according to traditional methods the keys of Payne et al. (1998) and Barnett et al. (2000) were used. *Trichosporon* spp. were identified by the key proposed by Middelhoven (2004), using non-traditional growth substrates like uric acid, some phenolics and polysaccharides. In case of doubt, the strains were identified by sequencing of the D1D2 region of 26S rDNA and the ITS region. Basidiomycetes were identified by Dr. Gloria Scorzetti of the University of Miami following the methods described by Scorzetti et al. (2002). Ascomycetes were identified by Dr. C.P. Kurtzman, Peoria, Illinois (Kurtzman and Robnett 2003).

Polysaccharides

Assimilation of polysaccharides was tested in GB medium containing 40 mM NH_4Cl at pH 5.5 and 6.5 (pH adjusted with KOH) under conditions described above, inulin and soluble starch at 5 g/l, dextran (Sigma, from *Leuconostoc*), pullulan (Sigma, filter-sterilized, from *Aureobasidium pullulans*), carboxymethyl cellulose (Sigma), chitin (Sigma, from crab shells), arabinogalactan (Sigma, from larch wood), gum xanthan (Sigma, from *Xanthomonas campestris*), galactomannan (Sigma, from locust bean) at 2.5 g/l, xylan (Sigma, from birch wood) at 10 g/l, the insoluble fraction of the latter was removed by centrifugation. These polysaccharides were tested at pH 6.5, but polygalacturonate (Sigma, potassium salt) and tannic acid (Sigma) were tested at 5 g/l GB medium at pH 5.5. All growth tests were conducted at 25 °C.

Results and discussion

The isolated and identified yeast strains are listed in Table 1. In March 2002, after a mild winter, three rotten wood species were sampled in the Wageningen forest. Encouraged by the great

Table 1. Yeast strains isolated from rotten wood samples.

From pine, *Pinus sylvestris* L., brownrot, March 2002
PiB-10 *Debaryomyces polymorphus* (Klöcker) Price et Phaff

From birch, *Betula pendula* Roth., whiterot, March 2002
BeW-1 *Trichosporon porosum* (Stautz) Middelhoven, Scorzetti et Fell
BeW-2 *Candida bertae* Ramírez et González CBS 10112

From beech, *Fagus sylvatica* L., whiterot, March 2002
FaW-11 *Cryptococcus fagi* Middelhoven et Scorzetti sp.nov. CBS 9964
FaW-12 *Candida bertae* Ramírez et González
FaW-13 *Leucosporidiella creatinivora* (Golubev) Sampaio, CBS 9965
FaW-14 *Leucosporidium scottii* Fell, Statzell, Hunter et Phaff, CBS 9250
FaW-15 *Aureobasidium pullulans* (de Bary) Arn.
FaW-21 *Trichosporon porosum* (Stautz) Middelhoven, Scorzetti et Fell

From pine, *Pinus sylvestris* L., brownrot, August 2002
PiB-71 *Candida* sp.nov. CBS 10106, NRRL Y-27775
PiB-37 *Pichia guillermondii* Wickerham, isolated at 37°C

From birch, *Betula pendula* Roth., whiterot, August 2002
BeW-71 *Yarrowia lipolytica* (Wickerham, Kurtzman et Herman) van der Walt et von Arx

From birch, *Betula pendula* Roth., brownrot, August 2002
BeB-71 *Trichosporon porosum* (Stautz) Middelhoven, Scorzetti et Fell
BeB-82 *Cryptococcus podzolicus* (Bab'eva et Reshetova) Golubev, CBS 9491
BeB-8371 *Kazachstania telluris* (van der Walt) Kurtzman CBS 10113, NRRL Y-27661, isolated at 37 °C, synonyms *Arxiozyma telluris* (van der Walt) van der Walt et Yarrow, *Saccharomyces telluris* van der Walt

From beech, *Fagus sylvatica* L., whiterot, August 2002
FaW-71 *Candida boleticola* Nakase CBS 10111, NRRL Y-27664

From beech, *Fagus sylvatica* L., brownrot, August 2002
FaB-81 *Trichosporon laibachii* (Windisch) Guého et M.Th.Smith
FaB-82 *Candida shehatae* var. *insectosa* Kurtzman CBS 10110, NRRL Y-27663
FaB-83 *Cryptococcus musci* Takashima, Sugita, Shinoda et Nakase, CBS 9492

From Douglas fir, *Pseudotsuga menziesii* (Mirb.) Franco, whiterot, August 2002
DoW-72 *Trichosporon porosum* (Stautz) Middelhoven, Scorzetti et Fell
DoW-73 *Candida ergatensis* Santa Maria CBS 10109, NRRL Y-27662

From *Robinia pseudoacacia* L., whiterot, August 2002
RoW-74 *Galactomyces geotrichum* (Butler et Petersen) Redhead et Mulloch, syn. *Geotrichum candidum* Link:Fr.

From *Robinia pseudoacacia* L., brownrot, August 2002
RoB-84 *Pichia anomala* (E.C.Hansen) Kurtzman NRRL Y-27776
RoB-86 *Trichosporon porosum* (Stautz) Middelhoven, Scorzetti et Fell

From spruce-fir, *Picea abies* L., whiterot, August 2002
SpaW-71 *Candida paludigena* Golubev et Blagodatskaya CBS 10108, NRRL Y-27666
SpaW-73 *Trichosporon porosum* (Stautz) Middelhoven, Scorzetti et Fell

From oak, *Quercus robur* L., brownrot, August 2002
QuB-82 *Candida* sp.nov. CBS 10107, NRRL Y-27665

variety of yeasts isolated, the experiment was repeated in August 2002, using more wood species. The wood samples were presumed to have undergone white-rot if cellulose fibres were still intact and brownrot when these were not visible anymore. The suspensions of most rotten wood samples yielded many yeast colonies. Apparently, yeasts are quite numerous, being the most common microbes next to bacteria. Weak acidity of the wood samples may have favoured development of the yeast flora. The pH-H_2O of suspensions of the samples was about 5.0; the pH-KCl 3.9–4.6.

Application of the identification keys in some cases unambiguously pointed to well-known yeast species. In other cases identification failed or was doubtful. These strains were identified by

sequencing of ribosomal DNA and were deposited in the CBS and/or the NRRL yeast culture collections. Two *Candida* spp. and one *Cryptococcus* sp. could not be identified. They represent novel species, the novel *Candida*'s to be described elsewhere and the basidiomycete as *Cryptococcus fagi* Middelhoven et Scorzetti in the present paper.

Two strains, viz. *Kazachstania telluris* BeB-8371 and *Leucosporidium scottii* FaW-14 grew only in complex media and failed to grow in Yeast Nitrogen Base and Yeast Carbon Base. The nature of their nutritional requirement is unknown. Identification in these cases completely depended on sequencing of ribosomal DNA. That of *K. telluris* BeB-8371 matched that of the type strain, but the ITS sequence of *L. scottii* FaW-14 was 3 base pairs different from that of the type strain of *L. scottii*, D1D2 regions being identical. Strain FaW-14 differed from the type strain of the closely related *Leucosporidiella creatinivora* by one base pair in ITS and one in D1D2. Both species are known as soil inhabitants.

Some ascomycetous species are known as inhabitants of rotten wood. *Candida bertae* has been isolated from two wood species in Chile (Ramírez and González 1984a). Strain BeW-2 failed to grow on 2-ketogluconate, but strain FaW-12 grew within a few days. All other characteristics of *C. bertae* listed by Barnett et al. (2000) were shown by both strains. There was no need to confirm this identification by rDNA sequencing. BeW-2 produced mainly budding yeast cells, but growth of FaW-12 was very mycelial. Strain FaW-71 was identified as *C. boleticola* (Nakase 1971b), a species isolated from rotten leaves and soil in Inner Mongolia and from a toadstool. Strains conspecific with *C. boleticola* had been isolated from rotten wood in Chile and had been described as *C. laureliae* and *C. ralunensis* (Ramírez and González 1984b). Strain FaW-71 differed from the species description by slow and delayed growth on 2-ketogluconate and by assimilation of creatinine (slow) and D-glucosamine as sole nitrogen sources. *C. ergatensis* DoW-73 differed from two other strains known of this species by absence of hyphae, by growth at 30 °C and by slow growth on melezitose. *C. ergatensis* Santa Maria (1971) has been isolated from beetles in Spain. *C. paludigena* SpaW-71 differed from the type strain CBS 8005 isolated from high-moor peat near Moscow (Golubev et al. 1981b) by growth on arbutin (slow), raffinose, xylitol (slow) and D-glucosamine as nitrogen source, and by failure to grow on 2-ketogluconate and D-glucuronate. Strain FaB-82 was identified by sequencing of rDNA as *C. shehatae* Buckley and van Uden (1967). It deviates from the species description by failure to grow on 5-ketogluconate and by slow assimilation of creatinine, D-glucosamine and D-tryptophan, nitrogen sources not assimilated by other strains of this species. More precisely, strain FaB-82 was identified as *C. shehatae* var. *insectosa* Kurtzman 1990 from which it deviates by slow growth on L-sorbose and L-arabinose instead of no growth by the two other strains known of this variety. Strains of *C. shehatae* have been isolated from soil, rotten wood in Chile and insects. Like other strains of this species, *C. shehatae* FaB-82 fermented D-xylose albeit slowly. Strain PiB-10 was identified by traditional methods as *Debaryomyces polymorphus*. Other strains of this species have been isolated from soil in several countries, a toadstool and an ant-hill. Identification of *Aureobasidium pullulans* FaW-15 was confirmed by sequencing of rDNA by Dr. G.S. de Hoog. *Galactomyces geotrichum* RoW-74, *Pichia guillermondii* PiB-37 and *Yarrowia lipolytica* BeW-71 were identified by traditional methods. They represent species very common in nature, isolated from soil and other habitats.

Most of the ascomycetous yeast strains isolated assimilated *n*-hexadecane (Table 3) when tested in slant cultures according to Markovetz and Kallio (1964, cf. Middelhoven 2001).

Of the basidiomycetous yeast strains isolated, only *Cryptococcus musci* FaB-83 assimilated *n*-hexadecane, conform the type strain of this species (Takashima et al. 2001). Strain FaB-83 differed from the type strain CBS 8899 by a lower maximum growth temperature, 30 °C rather than 33–34 °C, and by failure to assimilate soluble starch and D-galacturonic acid. *C. fagi* FaW-11 showed weak and delayed growth on *n*-hexadecane. *Cryptococcus podzolicus* BeB-82 differed from other strains of this species (Golubev 1981) by delayed and weak growth on D-glucuronic acid and by growth at 30 °C, a temperature not permitted by the other strains of this soil-borne yeast species. *Leucosporidiella (Rhodotorula) creatinivora* FaW-13 differed in some base sequences from the type strain CBS 8620, isolated by Golubev (1998) from permafrost soil. Some physiological data also differed. Presented as FaW-13 vs. CBS 8620: D-xylose DW/+, L-rhamnose +/−, galactitol +/−, D-glucuronate DW/+,

citrate DW/+, propane-2,3-diol −/+. A comparison with the physiological data of the closely related species *Leucosporidium scottii* (Fell et al. 1969), compiled by Barnett et el. (2000), based on a study of many strains, one from a rotten tree trunk in Chile included, reveals, presented as FaW-13 vs. species: D-xylose DW/+, citrate DW/+, propane-2,3-diol −/+, D-creatine as sole nitrogen source +/−. *L. scottii* is variable for many characters, but negative for creatine. For this reason strain FaW-13 was accommodated in *L. creatinivora*. *Trichosporon laibachii* is a common soil inhabitant. In this study it is represented by strain FaB-81, that showed all characteristics compiled by Guého et al. (1992), Barnett et al. (2000) and by Middelhoven (2004). *Trichosporon porosum* was isolated from six rotten wood samples. It is a very common soil inhabitant (Middelhoven et al. 2001), but strains are often not recognized as *Trichosporon* species because of poor development of arthroconidia. All six isolates showed the characteristics of the species according to Middelhoven et al. (2001) and Middelhoven (2004), but growth on L-phenylalanine as carbon and nitrogen source was very delayed or even negative. For all security, identification of one strain, FaW-21, was confirmed by sequencing of rDNA.

In addition to the three novel species, three isolated strains belonged to species hitherto only known by their type strain. This was the case with *C. paludigena* SpaW-71 and *C. musci* FaB-83. Of two other species only two strains were known. *C. bertae* BeW-2 and FaW-12 and *C. ergatensis* DoW-73 contribute to our knowledge of these rare species. The other isolated species were represented in culture collections by several strains.

Table 2. Assimilation of polysaccharides by yeast strains isolated from rotten wood samples.

Polysaccharide	Strain	Inu	Sta	Pul	Dex	Xyl	PGA	GM	Tan
Ascomycetes									
Aureobasidium pullulans	FaW-15	−	+	D	−	+	+	W	−
Candida bertae	FaW-12	−	−	+	−	DW	−	−	−
Candida boleticola	FaW-71	−	−	−	−	+	−	−	−
Candida ergastensis	DoW-73	−	−	−	−	+	−	−	−
Candida paludigena	SpaW-71	−	−	−	−	D	−	−	−
Candida shehatae var. *insectosa*	FaB-82	−	D	+	−	D	−	−	−
Candida sp.nov.	QuB-82	−	−	−	−	D	−	−	−
Candida sp.nov.	PiB-71	−	−	D	−	D	−	−	−
Debaryomyces polymorphus	PiB-10	+	−	D	−	+	−	−	−
Galactomyces geotrichum	RoW-74	−	−	D	−	D	+	−	−
Kazachstania telluris	BeB-8371	no growth in YNB and GB							
Pichia anomala	RoB-84	−	+	−	−	D	−	D	−
Pichia guillermondii	PiB-37	−	−	D	−	D	−	−	−
Yarrowia lipolytica	BeW-71	−	−	−	−	−	−	−	−
Yeast-like fungus	RoB-84	−	+	−	−	D	−	D	−
Basidiomycetes									
Cryptococus fagi sp.nov.	FaW-11	DW	+	−	−	−	−	−	+
Cryptococcus musci	FaB-83	−	−	D	−	−	−	+	+
Cryptococcus podzolicus	BeB-82	−	−	+	+	+	+	+	−
Leucosporidiella creatinivora	FaW-13	−	−	+	−	−	+	+	+
Leucosporidium scottii	FaW-14	No growth in YNB and GB							
Trichosporon laibachii	FaB-81	−	+	−	−	+	+	+	−
Trichosporon porosum	BeW-1	−	+	D	+	+	+	+	+
Trichosporon porosum	BeB-74	DW	+	D	+	+	+	+	+
Trichosporon porosum	DoW-72	−	+	−	+	+	+	+	+
Trichosporon porosum	RoB-86	−	+	D	+	+	+	+	+
Trichosporon porosum	SpaW-73	+	+	−	+	+	+	+	+
Trichosporon porosum	FaW-21	DW	+	−	+	+	+	+	+

+, growth within 8 days; D, 8–15 days required; −, no growth within 15 days; Inu, inulin 5 g/l, pH 6.5; Sta, soluble starch, 5 g/l, pH 6.5; Pul, pullulan, 5 g/l, pH 6.5; Dex, dextran, 2.5 g/l, pH 6.5; Xyl, xylan, 10 g/l, pH 6.5, soluble fraction only; PGA, sodium polygalacturonate, 5 g/l, pH 5.5; GM, galactomannan; Tan, tannic acid, 5 g/l, pH 5.5. No growth was observed on carboxymethyl cellulose, colloidal chitin, arabinogalactan and gum xanthan.

Assimilation of polysaccharides and other non-traditional carbon sources

In Table 2 assimilation of some polysaccharides by all strains is recorded. No growth was observed in media with carboxymethyl cellulose, chitin, arabinogalactan and gum xanthan. It is evident that ascomycetous yeasts displayed little activity to the assimilable polysaccharides. The yeastlike fungus *A. pullulans* assimilated starch, xylan and polygalacturonate. *D. polymorphus* grew well on inulin and xylan and *G. geotrichum* on polygalacturonate. *C. bertae* and *C. shehatae* are notable for growth on pullulan. Other growth responses were absent, weak or delayed. Contrary to this, the basidiomycetous strains tested assimilated many polysaccharides. *C. podzolicus* and both *Trichosporon* species, *T. porosum* in particular, grew well on most of the administered compounds. Growth responses to these separated saprotrophic *Trichosporon* species (Middelhoven 2004). Dextran, galactomannan and tannic acid were readily assimilated by basidiomycetes only.

Assimilation of some aliphatic and phenolic compounds (Table 3) correlates with the phylogenetic position of the yeast species. Two ascomycetous true yeasts readily growing at the expense of these compounds, viz. *C. bertae* and *C. paludigena*, belong to the *Stephanoascus*/*Blastobotrys* clade (Kurtzman and Robnett 1998) and are related to a group of closely related species that assimilate various purines, amines and branched-chain aliphatic compounds (Middelhoven and Kurtzman 2003). *Y. lipolytica* belongs to the *Metschnikowia* clade and failed to grow on phenolic compounds, like other identified *Candida*,

Table 3. Assimilation of *n*-hexadecane, *n*-butylamine, uric acid and some phenolic compounds by yeast strains isolated from rotten wood samples.

Carbon compound	Strain	C16	BuA	Uri	HQ	Res	3OB	4OB	4OP	4OC
Ascomycetes										
Aureobasidium pullulans	FaW-15	D	–	–	+	+	+	+	+	+
Candida bertae	BeW-2	+	–	–	+	+	+	–	–	–
Candida bertae	FaW-12	+	–	–	+	+	+	+	+	+
Candida boleticola	FaW-71	+	–	–	–	–	–	–	–	–
Candida ergatensis	DoW-73	+D	W	–	–	–	+	D	–	–
Candida paludigena	SpaW-71	–	D	–	+	+	+	+	+	+
Candida shehatae var. *insectosa*	FaB-82	+	W	–	–	–	–	–	–	–
Candida sp.nov.	QuB-82	–	–	+	–	–	+	–	–	–
Candida sp.nov.	PiB-71	–	–	+	+	+	+	–	+	–
Debaryomyces polymorphus	PiB-10	+	–	–	–	–	+	+	–	–
Galactomyces geotrichum	RoW-74	–	D	–	–	–	–	–	–	–
Kazachstania telluris	BeB-8371	No growth in YNB and GB								
Pichia guillermondii	PiB-37	+	W	–	–	–	–	–	–	–
Yarrowia lipolytica	BeW-71	+	+	–	–	–	–	–	–	–
Basidiomycetes										
Cryptococus fagi sp.nov.	FaW-11	DW	–	–	–	–	–	–	–	–
Cryptococcus musci	FaB-83	+	+	–	+	+	–	+	+	+
Cryptococcus podzolicus	BeB-82	–	–	–	–	+	–	–	–	–
Leucosporidiella creatinivora	FaW-13	–	+	–	+	+	+	+	+	+
Leucosporidium scottii	FaW-14	no growth in YNB and GB								
Trichosporon laibachii	FaB-81	–	+	+	+	+	+	+	+	+
Trichosporon porosum	BeW-1	–	+	–	+	+	+	+	+	+
Trichosporon porosum	BeB-74	–	+	–	+	+	+	+	+	+
Trichosporon porosum	DoW-72	–	+	–	+	+	+	+	+	+
Trichosporon porosum	RoB-86	–	+	–	+	+	+	+	+	+
Trichosporon porosum	SpaW-73	–	+	–	+	+	+	+	+	+
Trichosporon porosum	FaW-21	–	+	–	+	+	+	+	+	+

+, growth within 8 days; D, 8–15 days required; –, no growth within 15 days; C16, *n*-hexadecane; BuA, *n*-butylamine; Uri, uric acid; HQ, hydroquinone; Res, resorcinol; 3OB, 3-hydroxybenzoate; 4OB, 4-hydroxybenzoate; 4OP, 4-hydroxyphenylacetate; 4OC, 4-hydroxycinnamate. Uric acid and butylamine were tested in liquid GB medium, the other substrates on GB-ammonia agar slants.

Debaryomyces and *Pichia* species that belong to the *Debaryomyces*/*Lodderomyces* clade (Kurtman and Robnett 1998). However, *C. ergatensis* and *D. polymorphus* assimilated hydroxybenzoates. The undescribed *Candida* sp. PiB-71 assimilated several phenolics. Its phylogenetic position is yet unknown. Basidiomycetous yeasts that assimilated phenolics were found in the Trichosporonales (Middelhoven 1993, 2004) and in the *Microbotryum* clade. *L. creatinivora* FaW-13 belongs to the latter clade and assimilated many of the phenolic compounds tested (Table 3), conform *L. scottii* and some *Rhodotorula* spp. (Middelhoven 1993). Sampaio (1999) found that Tremellales generally do not assimilate aromatic compounds. *C. fagi* FaW-11 and *C. podzolicus* BeB-82 confirmed this, but *C. musci* FaB-83, an other member of the Tremellales (Takashima et al. (2001) grew well on several phenolics (Table 3).

Is Leucosporidiella creatinivora *an anamorph of* Leucosporidium scottii?

The type strains of *L. scottii* and *L. creatinivora* differ by only one base pair in D1D2 and four in ITS (G. Scorzetti, personal communication). Strain FaW-13 was tentatively identified as *L. creatinivora*, like strain UG-20 (CBS 9490) that was isolated from soil by enrichment on urotropine (hexamethylenetetramine) as sole nitrogen source (unpublished). A comparative study of the type strains of both species, viz. *L. scottii* CBS 5930 and *L. creatinivora* CBS 8602, and strains FaW-13 and UG-20 revealed far-reaching physiological similarity, creatine assimilation being the only distinguishing character. All four strains assimilated L-phenylalanine, resorcinol, salicylic acid, vanillic acid, 4-hydroxyphenylacetic acid, 3-hydroxycinnamic acid, ferulic acid and polygalacturonate as sole carbon sources, but failed to grow on uric acid, ethanolamine, L-4-hydroxyproline, soluble starch, pullulan, dextran, xylan, galactomannan, orcinol and phloroglucinol. These characters distinguish *Trichosporon* species (Middelhoven 2003, 2004). Close phylogenetic relationship, the existence of intermediate genomes and great physiological similarity render the introduction of *L.* (*Rhodotorula*) *creatinivora* questionable, the species may better be considered as an anamorph of *L. scottii*. The final proof will be found in mating studies of sexually competent strains of both species and by DNA/DNA reannealing.

Description of *C. fagi* sp.nov.

Latin diagnosis of C. fagi Middelhoven *et Scorzetti*

In medio liquido dextrosum et peptonum et extractum levidinis et extractum malti post 3 dies 25 °C cellulae globosae ad ovoideae (3.7–6.2×6.2–12.5 μm), singulae vel binae, hyphae et pellicula non formantur. Sedimentum album formatur, quod etiam post hebdomades 4 adest. Cultura in agaro PDA dicto post dies 3 albida, butyrosa, nitida, non elevata, margo integer; post hebdomades 4, 20 °C, eadem forma. In agaro *Zeae maydis* confecto post dies 5, 20 °C, nec mycelium nec pseudomycelium formantur. Fermentatio nulla. D-Glucosum, D-galactosum, L-sorbosum (lente), D-glucosaminum (lente), *N*-acetyl-D-glucosaminum, D-ribosum, L-arabinosum, D-arabinosum (lente) L-rhamnosum, sucrosum, maltosum, trehalosum, methyl-á-D-glucosum, melibiosum, lactosum, raffinosum, melezitosum, xylitolum (lente), glucitolum, mannitolum, galactitolum, myo-inositolum, D-gluconolactonum, acidum gluconicum, acidum 2-ketogluconicum, acidum 5-ketogluconicum, acidum galacturonicum, acidum succinicum, ethanolum (lente) assimilantur. Cellobiosum, arbutinum, salicinum, inulinum, amylum solubile, glycerolum, erythritolum, ribitolum, L-arabitolum, methanolum, propano-1,2-diolum, butano-2,3-diolum, acidum quinicum non assimilantur. Aethylaminum, natrii nitritum, D-glucosaminum assimilantur, neque kalii nitratum, L-lysinum, cadaverinum, creatinum, creatininum, imidazolum. Thiaminum externum crescentiae necessarium. Reactio diazonii coerulei B positivum. Ureum finditur. 30 °C crescit neque 32 °C. Typus CBS 9964, isolatus ex *Fagus sylvatica* in Wageningen, Neerlandia, lyophilus praeservatus in collectione zymotica Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands.

Characteristics of C. fagi *Middelhoven et Scorzetti*

After 3 days growth in liquid medium containing glucose (1%, w/v), peptone (0.5%, w/v), yeast extract (0.3%, w/v), malt extract (0.3%, w/v) at

Table 4. Characteristics of *Cryptococcus fagi* sp.nov.

Assimilation of carbon and nitrogen compounds
C1 D-Glucose +
C2 D-Galactose +
C3 L-Sorbose D
C4 D-Glucosamine D
C5 D-Ribose + D
C6 D-Xylose +
C7 L-Arabinose +
C8 D-Arabinose D
C9 L-Rhamnose +
C10 Sucrose +
C11 Maltose +
C12 α,α′-Trehalose +
C13 Me-α-D-Glucoside + D
C14 Cellobiose −
C15 Salicin −
C16 Arbutin −
C17 Melibiose +
C18 Lactose + D
C19 Raffinose +
C20 Melezitose +
C21 Inulin −
C22 Starch −, DW
C23 Glycerol −
C24 Erythritol −
C25 Ribitol −
C26 Xylitol + D
C27 L-Arabinitol −
C28 D-Glucitol + D
C29 D-Mannitol +
C30 Galactitol +
C31 myo-Inositol +
C32 D-Gluconolactone + D
C33 2-Keto-D-gluconate +
C34 5-Keto-D-gluconate +
C35 D-Gluconate +
C36 D-Glucuronate DW
C37 D-Galacturon. ac. +
C38 DL-Lactate DW
C39 Succinate + D
C40 Citrate −D
C41 Methanol −
C42 Ethanol D
C43 Propane-1,2-diol −
C44 Butane-2,3-diol −
C45 Quinic acid −
C46 D-Glucarate V
C47 D-Galactonate V
C48 Palatinose +
C49 Levulinate −
C50 L-Malic acid +
C51 L-Tartaric acid −
C52 D-Tartaric acid +
C53 meso-Tartaric acid −
C54 Galactaric acid + D
C55 Uric acid −
C56 Gentobiose -,D
C57 Ethylene glycol −
C58 Tween 40 ?
C59 Tween 60 −
C60 Tween 80 −
N1 Nitrate −
N2 Nitrite + D
N3 Ethylamine + D
N4 L-Lysine DW
N5 Cadaverine −
N6 Creatine −
N7 Creatinine −
N8 D-Glucosamine +
N9 Imidazole −
N10 D-Tryptophan + D
N11 D-Proline D
N12 Putrescine D
Miscellaneous
O1 0.01% Cycloheximide −
O2 0.1% Cycloheximide −
O3 1% Acetic acid ?
O6 10% NaCl −
O7 16% NaCl −
O8 Growth pH 3 +
O9 Growth pH 9.5 −
M1 Amyloids −, D very light blue
M3 Urease +
M4 Diazonium Blue B +
N-Acetyl-D-glucosamine +
n-Hexadecane DW
Growth temperature 30 °C +, 32 °C−
Vitamin required: Thiamine

25 °C cells are globose to ovoid (3.7–6.2×6.2–12.5 μm). A sediment and a ring are present, but a pellicle is not formed. The slant culture on PDA after 3 days is of butyrous structure and is white and glistening. The appearance does not change over 4 weeks. Slide cultures on malt extract agar, PDA agar and Yeast Morphology agar did not show hyphae but globose to ovoid cells only. The type strain is CBS 9964 (JCM 13614). Physiological characteristics of strain CBS 9964 were listed in Table 4. Its phylogenetic position is depicted in Figure 1.

The novel species *C. fagi* belongs to the Tremellales. Some *Tremella* and *Fibulobasidium* species are the closest relatives (Figure 1). *Cryptococus skinneri* is also a member of this clade. *C. fagi* is characterized by assimilation of many carbohydrates, polysaccharides included, but cellobiose and several polyols are not assimilated. Failure to assimilate phenolic compounds is a character common with most Tremellales.

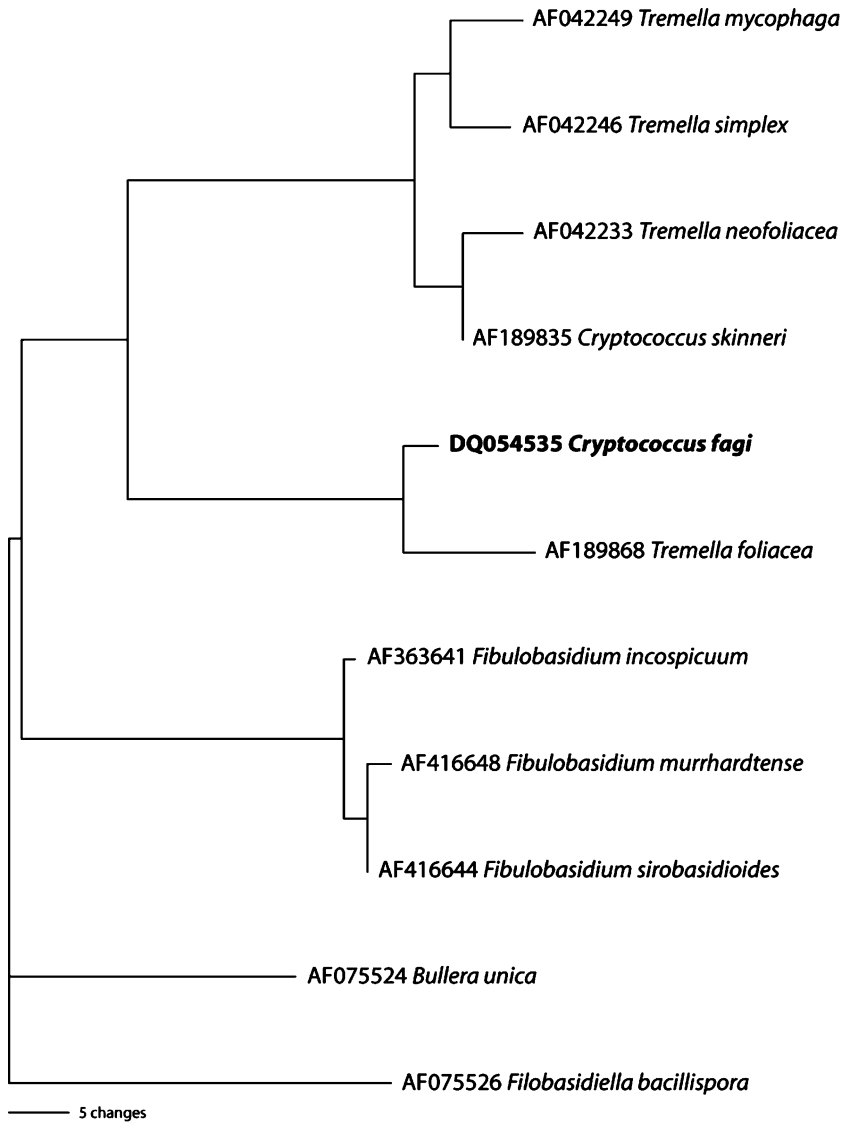

Figure 1. Single most parsimonious tree of *C. fagi* and related species based on PAUP 4.0 parsimony analysis (heuristic search, random step-wise addition, tree bisection-reconnection) from D1D2 sequences of the large subunit rDNA. *Bullera unica* and *Filobasidiella bacillospora* were used as an outgroup.

It can be seen (Figure 1) that *Tremella foliacea* (Persoon: Fries (1823) is the closest relative. This species is very common and is known as Brown Witch's Butter. This species is variable for many physiological characters, but all 8 strains studied failed to grow on α-methyl-sc d-glucoside, melibiose, lactose and raffinose (Barnett et al. 2000), compounds assimilated by *C. fagi* (Table 4). On the other hand, the novel species did not assimilate erythritol and ribitol, and showed weak and delayed growth on D-glucuronate, compounds supporting growth of *T. foliacea*. These growth tests clearly demonstrate that *C. fagi* is a hitherto undescribed species, well distinguishable from its closest relative.

Origin and deposits

Strain CBS 9964 (FaW-11) was isolated by W.J. Middelhoven from rotten beech wood (*Fagus sylvatica* L.) in Wageningen, The Netherlands in

March 2002. Nucleotide sequences of the D1D2 and ITS domains of the large subunit (26S) ribosomal DNA were deposited at Genbank by G. Scorzetti, accession numbers DQ054535 for D1D2 and DQ054534 for ITS.

Etymology

The epitet *fagi* refers to *Fagus sylvatica*, the tree from which the rotten wood sample originated.

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