Rhodotorula bloemfonteinensis sp. nov., *Rhodotorula eucalyptica* sp. nov., *Rhodotorula orientis* sp. nov. and *Rhodotorula pini* sp. nov., yeasts isolated from monoterpene-rich environments

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Recent rDNA sequencing of 25 isolates from a previous study, during which limonene-utilizing yeasts were isolated from monoterpene-rich environments by using 1,4-disubstituted cyclohexanes as sole carbon sources, led to the identification of four hitherto unknown *Rhodotorula* species. Analyses of the 26S rDNA D1/D2 region as well as the internal transcribed spacer (ITS) domain indicated that two isolates (CBS 8499^T and CBS 10736) were identical and were closely related to *Rhodotorula cycloclastica*, a previously described limonene-utilizing yeast. These novel isolates differed from known yeast species and could be distinguished from *R. cycloclastica* by standard physiological tests. The other three isolates represent three novel *Rhodotorula* species, closely related to *Sporobolomyces magnisporus*. These three species could also be distinguished from other *Rhodotorula* species by standard physiological tests. Based on these results, we suggest that the new isolates represent novel species, for which the names *Rhodotorula eucalyptica* sp. nov. (type strain CBS 8499^T = NRRL Y-48408^T), *Rhodotorula pini* sp. nov. (type strain CBS 10735^T = NRRL Y-48410^T), *Rhodotorula bloemfonteinensis* sp. nov. (type strain CBS 8598^T = NRRL Y-48407^T) and *Rhodotorula orientis* sp. nov. (type strain CBS 8594^T = NRRL Y-48719^T) are proposed. *R. eucalyptica* and *R. pini* can also utilize limonene.

Eucalyptus and coniferous trees are known sources of monoterpenes, including limonene, α -pinene and β -pinene (Cook, 1992). These compounds are known to be potential plant protectants, especially against insect attack. In addition, monoterpenes impart certain sensory qualities, including fragrance, to plants (King & Dickinson, 2003) and have found application in the fragrance, flavour and pharmaceutical industries (Pavlostathis & Misra, 1999; King & Dickinson, 2003). Several authors have demonstrated that yeasts are capable of biotransforming monoterpenes to yield products with desirable aromatic properties (Van Rensburg *et al.*, 1997; Pavlostathis & Misra, 1999; King & Dickinson, 2000, 2003). However, the isolation of yeasts with the ability to utilize monoterpenes

as sole carbon and energy sources has proven difficult, due to the toxicity of many of these compounds (Nguyen Thanh *et al.*, 2004). The latter authors reported that this problem can be overcome by using less toxic monoterpene analogues such as 1,4-disubstituted cyclohexanes as sole carbon sources for enrichments. In December 1995, they performed a series of isolation experiments using soil samples from eucalyptus plantations in Malaysia and Thailand and pine leaf litter from Bloemfontein, South Africa. Enrichments had been done using 1,4-cyclohexanedimethanol (0.1 % w/v), 1,4-cyclohexanedicarboxylic acid (0.1 % w/v) or 4-aminobenzoate (0.1 % w/v) as sole carbon sources. Fifteen isolates obtained from these enrichments were initially sent to the Centraalbureau voor Schimmelcultures (CBS) for identification, but only five could be identified to the species level based on physiological tests. These were identified as *Rhodotorula phillyla* (CBS 8554), *Rhodotorula glutinis* (CBS 8587, later reclassified by the CBS as *Rhodospiridium toruloides*), *Rhodotorula mucilaginosa* (CBS 8596), *Cryptococcus laurentii* (CBS 8593) and *Pichia subpelliculosa* (CBS 8595). Five of the strains that could not be identified to the species level

Abbreviation: ITS, internal transcribed spacer.

The GenBank/EMBL/DBJ accession numbers for the 26S rDNA D1/D2 domain and ITS region sequences of strains CBS 8499^T, CBS 10736, CBS 8598^T, CBS 10735^T and CBS 8594^T are respectively EU075183, EU075184, EU075187, EU075188 and HM559718 (D1/D2) and EU075185, EU075186, EU075189, EU075190 and HM559719 (ITS).

and that were able to utilize limonene vapours as a carbon source were investigated further. One of these was also identified as *R. philyla* (CBS 8589), and the others were described as members of three novel species, *Rhodotorula retinophila* (CBS 8446), *Rhodotorula cycloclastica* (CBS 8447, CBS 8448) and *Rhodotorula terpenoidalis* (CBS 8445) (Nguyen Thanh *et al.*, 2004). In this study, we subjected the remaining five unidentified isolates as well as a further 20 isolates from the 1995 isolations to rDNA sequencing.

The 26S rDNA D1/D2 region as well as the internal transcribed spacer (ITS) region was amplified by PCR and sequenced using primer pair NL1 (5'-GCATATCAATA-AGCGGAGGAAAAG-3') and NL4 (5'-GGTCCGTGTTT-CAAGACGG-3') (Kurtzman & Robnett, 1998) and primer pair ITS4 (5'-TCCTCCGCTTATTGATATGS-3') and ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') (White *et al.*, 1990), respectively. Sequence analyses were performed with reference to sequences obtained from the GenBank database. Sequence alignments were done using CLUSTAL_X 2.0.11 (Larkin *et al.*, 2007) and phylogenetic analyses were conducted with MEGA version 4 (Tamura *et al.*, 2007) using

neighbour-joining with Kimura's two-parameter distance measure. Confidence values were estimated from bootstrap analysis of 1000 replicates.

A summary of the 35 isolates identified during this and the previous study (Nguyen Thanh *et al.*, 2004) is given in Table 1. Twelve of the 21 basidiomycetous isolates are able to utilize limonene vapours as a carbon source, although growth on limonene was sometimes variable. None of the 14 ascomycetous isolates could grow on limonene as a sole carbon source, although most of them could utilize hexadecane.

Sequence comparison of the 26S rDNA D1/D2 and ITS regions of the previously unidentified isolates from enrichments containing 1,4-cyclohexanedimethanol and 1,4-cyclocyclohexanedicarboxylic acid with related sequences indicated that they belong to two previously unknown species in the order Erythrobasidiales, class Cystobasidiomycetes (Hibbett *et al.*, 2007), and are closely related to *Sporobolomyces magnisporus*. For the isolate obtained from pine litter from South Africa (CBS 10735^T), we propose the name *Rhodotorula pini* sp. nov. and, for the

Table 1. Isolates obtained from monoterpene-rich environments after use of 1,4-substituted cyclohexanes as carbon sources in enrichments

CBS numbers are given for isolates deposited with the Centraalbureau voor Schimmelcultures. Isolates that have not been deposited have TVN numbers. Limonene-utilizers are underlined.

Species	Isolate(s)	Source(s)*	Carbon source(s) for enrichment†
Ascomycetes			
<i>Debaryomyces hansenii</i>	TVN 287	PLBS	4-ABA
<i>Pichia anomala</i>	TVN 327	PLBS	4-ABA
<i>Pichia fermentans</i>	TVN 288	PLBS	1,4-CHDCA
<i>Pichia guilliermondii</i>	TVN 313, TVN 320, TVN 330, TVN 331, TVN 332, TVN 333, TVN 352, TVN354, TVN 358	PLBS, SEPT	4-ABA, 1,4-CHDCA
<i>Pichia</i> sp.	TVN 353‡	PLBS	1,4-CHDM
<i>Pichia subpelliculosa</i>	CBS 8595§	PLBS	1,4-CHDCA
Basidiomycetes			
<i>Cryptococcus laurentii</i>	CBS 8593§	PLBS	4-ABA
<i>Cryptococcus</i> sp.	TVN 297‡	SEPM	1,4-CHDM
<i>Occultifur externus</i>	CBS 8591, CBS 8597, CBS 8590	PLBS	1,4-CHDM
<i>Rhodotorula bloemfonteinensis</i> sp. nov.	CBS 8598 ^T	PLBS	4-ABA
<i>Rhodotorula cycloclastica</i>	<u>CBS 8447§</u> , <u>CBS 8448§</u> , <u>TVN336</u> , <u>TVN 340</u>	SEPT, PLBS	1,4-CHDCA, 1,4-CHDM
<i>Rhodotorula eucalyptica</i> sp. nov.	<u>CBS 8499^T</u> , <u>CBS 10736</u>	SEPM	1,4-CHDM
<i>Rhodospiridium toruloides</i>	CBS 8587§	SEPM	1,4-CHDCA
<i>Rhodotorula mucilaginosa</i>	CBS 8596§	PLBS	1,4-CHDM
<i>Rhodotorula orientis</i> sp. nov.	CBS 8594 ^T	SEPM	1,4-CHDCA
<i>Rhodotorula philyla</i>	<u>CBS 8589§</u> , <u>CBS 8554§</u>	PLBS	1,4-CHDM
<i>Rhodotorula pini</i> sp. nov.	<u>CBS 10735^T</u>	PLBS	1,4-CHDM
<i>Rhodotorula retinophila</i>	<u>CBS 8446§</u> , <u>TVN 351</u>	PLBS	1,4-CHDCA
<i>Rhodotorula terpenoidalis</i>	<u>CBS 8445§</u>	SEPM	1,4-CHDCA

*PLBS, Pine leaf litter, Bloemfontein, South Africa; SEPM, soil of a eucalyptus plantation, Malaysia; SEPT, soil of a eucalyptus plantation, Thailand.

†1,4-CHDCA, 1,4-Cyclohexanedicarboxylic acid; 1,4-CHDM, 1,4-cyclohexanedimethanol; 4-ABA, 4-aminobenzoate.

‡Still under investigation since, according to rDNA sequencing, the isolate represents a novel species.

§Described by Nguyen Thanh *et al.* (2004).

isolate obtained from the Malaysian eucalyptus plantation (CBS 8594^T), we propose the name *Rhodotorula orientis* sp. nov. Comparison of the D1/D2 and ITS regions of isolate CBS 8598^T, obtained from the pine litter enrichment containing 4-aminobenzoate as sole carbon source, with related sequences indicated that it belongs to another previously unknown species, also related to *S. magnisporus*. We propose the name *Rhodotorula bloemfonteinensis* sp. nov. for this isolate. Phylogenetic analysis using a combined dataset consisting of the D1/D2 and ITS regions supports the recognition of *R. pini*, *R. orientis* and *R. bloemfonteinensis* as novel species (Fig. 1). These three species can be distinguished physiologically from each

other and from *S. magnisporus*, as indicated in Table 2. Furthermore, *R. pini* is able to grow on limonene vapours.

Nagahama *et al.* (2006) defined six robust subgroups within the *Erythrobasidium* clade (Erythrobasidiales). However, they did not include *S. magnisporus* in their study. Phylogenetic analysis based on a combined dataset consisting of the 26S rDNA D1/D2 region and ITS domain support the six subgroups as defined by Nagahama *et al.* (2006), but suggest a seventh subgroup (Magnisporus), comprising the three novel species (*R. pini*, *R. orientis* and *R. bloemfonteinensis*) as well as *S. magnisporus*, with a high bootstrap value.

Fig. 1. Phylogenetic tree derived from neighbour-joining analysis based on a combined dataset composed of the 26S rDNA D1/D2 region and the ITS domain showing the placement of *Rhodotorula pini* sp. nov., *Rhodotorula orientis* sp. nov. and *Rhodotorula bloemfonteinensis* sp. nov. (within clade A) and *Rhodotorula eucalyptica* sp. nov. (within clade B) relative to related taxa. Accession numbers for sequences of the 26S rDNA D1/D2 region/ITS domain are given unless deposited under a single accession number. *Rhodotorula mucilaginosa* CBS 316^T was included to root the tree. In clade A, the subgroups defined by Nagahama *et al.* (2006) as well as our newly described seventh subgroup (Magnisporus) are indicated. Bootstrap percentages (from 1000 replicates) $\geq 50\%$ are shown. Bar, 2% nucleotide sequence divergence.

Table 2. Salient characteristics of *Rhodotorula pini* sp. nov., *Rhodotorula bloemfonteinensis* sp. nov., *Rhodotorula orientis* sp. nov. and *Sporobolomyces magnisporus*

Strains: 1, *R. pini* sp. nov. CBS 10735^T; 2, *R. bloemfonteinensis* sp. nov. CBS 8598^T; 3, *R. orientis* sp. nov. CBS 8594^T; 4, *S. magnisporus* FK135^T (data from Nakase *et al.*, 2003). +, Positive; –, negative; w, weak.

Characteristic	1	2	3	4
Assimilation of:				
Sorbose	+	–	+	+ /w
Cellobiose	+	+	+	–
Lactose	–	+	+	–
D-Arabinose	+	+	+	–
Citrate	+	–	+	+ /w
Nitrate	–	–	+	+
Nitrite	–	+	+	+
L-Lysine	+	–	+	–
Growth in vitamin-free medium	–	+	+	–

Based on the comparison of 26S rDNA D1/D2 and ITS regions, two of the previously unidentified limonene-utilizing isolates (CBS 8499^T and CBS 10736), obtained from soil from a Malaysian eucalyptus plantation, were found to be identical. Phylogenetic analysis using a combined dataset consisting of the D1/D2 and ITS regions (Fig. 1) indicated that they belong to a previously unknown species, closely related to the limonene-utilizing yeast *R. cycloclastica*, for which we propose the name *Rhodotorula eucalyptica* sp. nov. This newly described species and *R. cycloclastica* differ at 16 positions in the D1/D2 region and at 93 positions in the ITS domain. Phylogenetic analysis indicated that *R. cycloclastica* previously grouped with *Rhodotorula subericola* (Belloch *et al.*, 2007) but, with the addition of *R. eucalyptica*, the latter species now forms a defined subgroup together with *R. cycloclastica* (Fig. 1). *R. cycloclastica* and *R. eucalyptica* in turn belong to a larger group of limonene-utilizing *Rhodotorula* species within the class Microbotryomycetes (Hibbett *et al.*, 2007) which also includes *R. retinophila*, *R. terpenoidalis* and *R. philyla* (Nguyen Thanh *et al.*, 2004). However, no information exists on the ability of *Colacogloe peniophorae* or *R. subericola* to utilize limonene. *R. eucalyptica* can be distinguished physiologically from *R. cycloclastica* as indicated in Table 3.

All the other *Rhodotorula* isolates with the ability to utilize limonene belong within a specific clade related to *R. cycloclastica* (class Microbotryomycetes) (Nguyen Thanh *et al.*, 2004). *R. pini* is thus the first yeast from a distant clade (class Cystobasidiomycetes) that displays potential for growth on limonene, although growth on limonene vapours was variable in tests done on different occasions. The disubstituted cyclohexanes proved very successful for the isolation of novel *Rhodotorula* species, since 14 of the 35 isolates of which rDNA was sequenced represent nine novel species, of which seven belong to the genus

Table 3. Salient characteristics of *Rhodotorula eucalyptica* sp. nov. and its closest relative, *R. cycloclastica*

Data for *R. cycloclastica* CBS 8448^T were taken from Nguyen Thanh *et al.* (2004).

Characteristic	<i>R. eucalyptica</i> sp. nov. CBS 8499 ^T	<i>R. cycloclastica</i> CBS 8448 ^T
Assimilation of:		
D-Xylose	+	–
Sucrose	+	–
Maltose	+	–
Melezitose	+	–
Arbutin	+	–

Rhodotorula. Five of these novel *Rhodotorula* species represent limonene-utilizers.

Latin diagnosis of *Rhodotorula pini* Pohl, M. S. Smit et Albertyn sp. nov.

In medio liquido extracto fermenti et extracto malti confecto, post dies tres ad 25 °C, cellulae cylindricae (8.2–20.5 × 2.8–5.2 µm). Pseudohyphae formantur. Cellulae multipolariter gemmantes, racemosae. In agar extracto fermenti en extracto malti confecto, post dies septem ad 25 °C, cultura glabra ad farinata, margine integra, aurantiaca. Ballistosporae non formantur. In agar Dalmat pseudohyphae formantur. Non fermentat. Glucosum, trehalosum, melezitosum (lente), N-acetylglucosaminum, glycerolum, erythritolum, mannitolium, salicinum, gluconatum, lactatum, succinatum, citratum, glucuronatum, arbutinum et limonenum (variabile) assimulantur. Galactosum, sorbosum, sucrosolum, maltosum, cellobiosum, lactosum, melibiosum, raffinolum, inulinum, amyllum solubile, xylosum, L-arabinosum, D-arabinosum, ribosum, rhamnosum, D-glucosaminum, ethanolum, methanolum, ribitolium, galactitolium, glucitolium, xylitolium, arabinitolum, α-methyl-D-glucosidum, galacturonatum, inositolium, propan-1,2-diolum, butan-2,3-diolum et hexadecanum non assimilantur. L-Lysinium et cadaverinum assimilantur neque nitratum, nitritum, creatinum, creatinum, imidazolium et ethylaminum. Ureum finditur. Materia amyloidea non formatur. Vitaminum externum crescentiae necessarium. Crescit in medio 0.01 % cycloheximidum continente. In medio 0.1 % cycloheximidum vel 1 % acidum aceticum vel 50 % glucosum continente non crescit. Non crescit ad 37 °C. Typus praeservatus in collectione zymotica Centraalbureau voor Schimmelcultures, Utrecht, Neerlandia (holotypus lyophilisatus CBS 10735^T) et in collectione zymotica Agricultural Research Services United States Department of Agriculture, Peoria, Illinois (isotypus NRRL Y-48410^T).

Description of *Rhodotorula pini* Pohl, M. S. Smit et Albertyn sp. nov.

Rhodotorula pini (pi'ni. N.L. gen. n. *pini* of *Pinus*, referring to the association of this yeast with trees of the genus *Pinus*).

In YM broth (1^{-1} : 3 g yeast extract, 3 g malt extract, 5 g peptone and 10 g glucose) after 3 days of growth at 25 °C, cells are elongated ($8.2\text{--}20.5 \times 2.8\text{--}5.2 \mu\text{m}$) (Fig. 2a), often forming pseudohyphae. Multipolar budding is observed and cells occur as clusters. On YM agar after 7 days of growth at 25 °C, colonies are smooth to slightly powdery with entire margins and orange in colour. No ballistospores are observed on cornmeal agar or YM agar. In Dalmat plate cultures, pseudohyphae can be observed. No fermentation is observed. The yeast is able to assimilate D-glucose, trehalose, melezitose (weakly), N-acetylglucosamine, glycerol, erythritol, mannitol, salicin, D-gluconate, lactate, succinate, citrate, glucuronate, arbutin and limonene (variable), but not galactose, sorbose, sucrose, maltose, cellobiose, lactose, melibiose, raffinose, inulin, soluble starch, xylose, L- or D-arabinose, ribose, rhamnose, D-glucosamine, ethanol, methanol, ribitol, galactitol, glucitol, xylitol, arabinitol, α -methyl D-glucoside, galacturonate, inositol, propane-1,2-diol, butane-2,3-diol or hexadecane. L-Lysine and cadaverine are utilized as nitrogen sources, but not nitrate, nitrite, creatinine, creatine, imidazole or ethylamine. The yeast

hydrolyses urea, but no extracellular starch is produced. Vitamins are necessary for growth. Growth is observed in the presence of 0.01 % cycloheximide, but not in the presence of 0.1 % cycloheximide, 1 % acetic acid or 50 % glucose. No growth is observed at 37 °C.

The type strain is deposited in the yeast culture collection of the Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands, as CBS 10735^T, and a copy has been deposited in the yeast culture collection of the Agricultural Research Services, United States Department of Agriculture, Peoria, Illinois, USA (NRRL Y-48410^T).

Latin diagnosis of *Rhodotorula bloemfonteinensis* Pohl, M. S. Smit et Alvertyn sp. nov.

In medio liquido extracto fermenti et extracto malti confecto, post dies tres ad 25 °C, cellulae ovoidae ($4.1\text{--}6.3 \times 2.4\text{--}3.0 \mu\text{m}$). Cellulae polariter gemmantes, singulae vel binae cohaerentes. In agar extracto fermenti et extracto malti confecto, post dies septem ad 25 °C, cultura glabra, salmona,

Fig. 2. Differential interference contrast micrographs of vegetative cells of *Rhodotorula pini* sp. nov. CBS 10735^T (a), *Rhodotorula bloemfonteinensis* sp. nov. CBS 8598^T (b), *Rhodotorula orientis* sp. nov. CBS 8594^T (c) and *Rhodotorula eucalyptica* sp. nov. CBS 8499^T (d) following growth in YM broth for 3 days at 25 °C. Bars, 10 μm .

marginē integra. Ballistosporae non formantur. In agarō Dalmau nec pseudohyphae nec hyphae formantur. Non fermentat. Glucosum, sucrosum, maltosum, cellobiosum, trehalosum, lactosum, melibiosum, raffinolum, melezitolum, amyllum solubile, D-arabiosum, ribosum (lente), ethanolum (lente), glycerolum, ribitolum, mannitolum (lente), glucitolum (lente), salicinum, gluconatum, lactatum, succinatum, glucuronatum, inositolum (lente) et arbutinum (lente) assimilantur. Galactosum, sorbosum, inulinum, xylosum, L-arabiosum, rhamnosum, glucosaminum, N-acetylglucosaminum, methanolum, erythritolum, galactitolum, xylitolum, arabinitolum, α -methyl-D-glucosidum, citratum, galacturonatum, propan-1,2-diolum, butan-2,3-diolum, hexadecanum et limonenum non assimilantur. Nitritum assimilantur neque nitratum, creatininum, creatinum, imidazolium, L-lysinum et ethylaminum. Urea non finditur. Materia amyloidea non formatur. Vitaminum externum crescentiae non necessarium. Crescit in medio 0.1 % cycloheximidum continente. In medio 1 % acidum aceticum vel 50 % glucosum continente non crescit. Non crescit ad 37 °C. Typus praeservatus in collectione zymotica Centraalbureau voor Schimmelcultures, Utrecht, Neerlandia (holotypus lyophilisatus CBS 8598^T) et in collectione zymotica Agricultural Research Services United States Department of Agriculture, Peoria, Illinois (isotypus NRRL Y-48407^T).

Description of *Rhodotorula bloemfonteinensis* Pohl, M. S. Smit et Albertyn sp. nov.

Rhodotorula bloemfonteinensis (bloem.fon.tei.nen'sis. N.L. fem. adj. *bloemfonteinensis* referring to Bloemfontein, South Africa, where the type strain was isolated).

In YM broth after 3 days of growth at 25 °C, cells are ovoid (4.1–6.3 × 2.4–3.0 µm) (Fig. 2b). Unipolar budding is observed and cells occur singly or as parent–bud pairs. On YM agar after 7 days of growth at 25 °C, colonies are smooth with entire margins and salmon-coloured. No ballistospores are observed on cornmeal agar or YM agar. In Dalmau plate cultures, no pseudohyphae or true hyphae can be observed. No fermentation is observed. The yeast is able to assimilate glucose, sucrose, maltose, cellobiose, trehalose, lactose, melibiose, raffinose, melezitose, soluble starch, D-arabinose, ribose (weakly), ethanol (weakly), glycerol, ribitol, mannitol (weakly), glucitol (weakly), salicin, D-gluconate, lactate, succinate, glucuronate, inositol (weakly) and arbutin (weakly), but not galactose, sorbose, inulin, xylose, L-arabinose, rhamnose, D-glucosamine, N-acetylglucosamine, methanol, erythritol, galactitol, xylitol, arabinitol, α -methyl D-glucoside, citrate, galacturonate, propane-1,2-diol, butane-2,3-diol, hexadecane or limonene. Nitrite is utilized as a nitrogen source, but not nitrate, creatinine, creatine, imidazole, L-lysine or ethylamine. The yeast does not hydrolyse urea and no extracellular starch is produced. Vitamins are not necessary for growth. Growth is observed in the presence of 0.1 % cycloheximide, but not in the presence of 1 % acetic acid or 50 % glucose. No growth is observed at 37 °C.

The type strain is strain CBS 8598^T (=NRRL Y-48407^T).

Latin diagnosis of *Rhodotorula orientis* Pohl, M. S. Smit et Albertyn sp. nov.

In medio liquido extracto fermenti et extracto malti confecto, post dies tres ad 25 °C, cellulae rotundae (3.6–7.0 µm). Cellulae polariter gemmantes, singulae vel binae cohaerentes. In agarō extracto fermenti et extracto malti confecto, post dies septem ad 25 °C, cultura glabra, salmonea, marginē integra. Ballistosporae non formantur. In agarō Dalmau nec pseudohyphae nec hyphae formantur. Non fermentat. Glucosum, sucrosum, maltosum, cellobiosum, sorbosum, trehalosum, lactosum, melibiosum, raffinolum (lente), melezitolum, xylosum, amyllum solubile (lente), D-arabiosum, L-arabiosum, ribosum, ethanolum, glycerolum, mannitolum (lente), erythritolum, glucitolum, arabinitolum, salicinum, α -methyl-D-glucosidum, gluconatum, lactatum, succinatum, citratum, glucuronatum N-acetylglucosaminum, propan-1,2-diolum, et arbutinum (lente) assimilantur. Galactosum, inulinum, rhamnosum, glucosaminum, methanolum, ribitolum, galactitolum, xylitolum, inositolum, galacturonatum, butan-2,3-diolum, hexadecanum et limonenum non assimilantur. Nitritum nitratum, cadaverinum, L-lysinum et ethylaminum assimilantur neque creatininum, creatinum et imidazolium. Urea non finditur. Materia amyloidea non formatur. Vitaminum externum crescentiae non necessarium. Crescit in medio 0.01 % cycloheximidum continente. In medio 1 % acidum aceticum vel 50 % glucosum continente non crescit. Non crescit ad 37 °C. Typus praeservatus in collectione zymotica Centraalbureau voor Schimmelcultures, Utrecht, Neerlandia (holotypus lyophilisatus CBS 8594^T) et in collectione zymotica Agricultural Research Services United States Department of Agriculture, Peoria, Illinois (isotypus NRRL Y-48719^T).

Description of *Rhodotorula orientis* Pohl, M. S. Smit et Albertyn sp. nov.

Rhodotorula orientis (o.ri.en'tis. L. fem. adj. *orientis* eastern, referring to eastern Malaysia, where the type strain was isolated).

In YM broth after 3 days of growth at 25 °C, cells are round (diameter 3.6–7.0 µm) (Fig. 2c). Unipolar budding is observed and cells occur singly or as parent–bud pairs. On YM agar after 7 days of growth at 25 °C, colonies are smooth with entire margins and salmon-coloured. No ballistospores are observed on cornmeal agar or YM agar. In Dalmau plate cultures, no pseudohyphae or hyphae can be observed. No fermentation is observed. The yeast is able to assimilate glucose, sucrose, maltose, cellobiose, sorbose, trehalose, lactose, melibiose, raffinose (weakly), melezitose, xylose, soluble starch (weakly), D- and L-arabinose, ribose, ethanol, glycerol, mannitol (weakly), erythritol, glucitol, arabinitol, salicin, α -methyl D-glucoside, D-gluconate, lactate, succinate, citrate, glucuronate, N-acetylglucosamine, propane-1,2-diol and arbutin (weakly), but not

galactose, inulin, rhamnose, D-glucosamine, methanol, ribitol, galactitol, xylitol, inositol, galacturonate, butane-2,3-diol, hexadecane or limonene. Nitrite, nitrate, cadaverine, L-lysine and ethylamine are utilized as nitrogen sources, but not creatinine, creatine or imidazole. The yeast does not hydrolyse urea and no extracellular starch is produced. Vitamins are not necessary for growth. Growth is observed in the presence of 0.01 % cycloheximide, but not in the presence of 1 % acetic acid or 50 % glucose. No growth is observed at 37 °C.

The type strain is CBS 8594^T (=NRRL Y-48719^T).

Latin diagnosis of *Rhodotorula eucalyptica* Pohl, M.S. Smit et Albertyn sp. nov.

In medio liquido extracto fermenti et extracto malti confecto, post dies tres ad 25 °C, cellulae ovoidae ad cylindricae (4.0–6.0 × 4.0–8.2 µm). Cellulae polariter gemmantes, binae cohaerentes. In agar extracto fermenti et extracto malti confecto, post dies septem ad 25 °C, cultura aquosa mucosescens vel glutinosescens, crenea, margine integra. In agar Dalmau nec pseudohyphae nec hyphae formantur. Non fermentat. D-Glucosum, D-glucosaminum, D-xylosum, sucrosum, maltosum, trehalosum, α-methyl-D-glucosidum (lente), arbutinum, melezitotum, ribitolum, xylitolum, D-glucitolum, D-mannitolum, succinatum, citratum, decanum, hexadecanum et limonenum (variabile) assimilantur. D-Galactosum, L-sorboseum, D-ribosum, L-arabinosum, D-arabinosum, rhamnosum, cellobiosum, salicinum, melibiosum, lactosum, raffinotum, inulinum, amyllum solubile, glycerolum, erythritolum, ribitolum, L-arabinitolum, galactitolum, inositolum, D-glucuronatum, D-glucuronatum, D-galacturonatum, DL-lactatum, propan-1,2-diolum, butan-2,3-diolum non assimilantur. Crescit in ethanolo neque methanolo. L-Lysinum (lente) et ethylaminum assimilantur neque nitratum, nitritum, creatininum, creatinum, cadaverinum et imidazolium. Ureum finditur. Materia amyloidea non formatur. Vitaminum externum crescentiae non necessarium. In medio 0.01 % cycloheximidum, 1 % acidum aceticum, 50 % glucosum continente non crescit. Non crescit ad 37 °C. Typus praeservatus in collectione zymotica Centraalbureau voor Schimmelcultures, Utrecht, Neerlandia (holotypus lyophilisatus CBS 8499^T) et in collectione zymotica Agricultural Research Services United States Department of Agriculture, Peoria, Illinois (isotypus NRRL Y-48408).

Description of *Rhodotorula eucalyptica* Pohl, M. S. Smit et Albertyn sp. nov.

Rhodotorula eucalyptica (eu.ca.lyp'ti.ca. N.L. fem. adj. *eucalyptica* of *eucalyptus*, referring to the association of this yeast with *eucalyptus* plantations).

In YM broth after 3 days of growth at 25 °C, cells are ovoid to elongate (4.0–6.0 × 4.0–8.2 µm) (Fig. 2d). Polar budding is observed and cells occur as parent–bud pairs. On YM agar after 7 days at 25 °C, colonies are mucoid to viscous with entire margins and cream coloured. In Dalmau plate

cultures, no pseudohyphae or true hyphae can be observed. No fermentation is observed. The yeast is able to assimilate D-glucose, D-glucosamine, D-xylose, sucrose, maltose, trehalose, α-methyl D-glucoside (weakly), arbutin, melezitotum, ribitol, xylitol, D-glucitol, D-mannitol, succinate, citrate, decane, hexadecane and limonene (variable), but not D-galactose, L-sorbose, D-ribose, L- or D-arabinose, rhamnose, cellobiose, salicin, melibiose, lactose, raffinose, inulin, starch, glycerol, erythritol, ribitol, L-arabinitol, galactitol, inositol, D-gluconate, D-glucuronate, D-galacturonate, DL-lactate, propane-1,2-diol or butane-2,3-diol. Growth is observed in ethanol, but not in methanol. L-Lysine (weakly) and ethylamine are utilized as nitrogen sources, but not nitrate, nitrite, creatinine, creatine, cadaverine or imidazole. The yeast hydrolyses urea but no extracellular starch is produced. Vitamins are not necessary for growth. No growth is observed in the presence of 0.01 % cycloheximide, 1 % acetic acid or 50 % glucose. No growth is observed at 37 °C.

The type strain is CBS 8499^T (=NRRL Y-48408^T).

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