Cryptococcus cyanovorans sp. nov., a basidiomycetous yeast isolated from cyanide-contaminated soil

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Eighteen yeast strains were isolated and identified from cyanide-contaminated soil in South Africa. According to sequence-based analyses using the D1/D2 region of the large ribosomal subunit and ITS region, three of these strains were found to be identical and represent a novel species. Phylogenetic analysis based on the combined dataset of the D1/D2 and ITS regions revealed a grouping with *Cryptococcus curvatus*, representing a defined clade (Curvatus) in the order Trichosporonales. The three strains were demarcated from *Cryptococcus curvatus* by standard physiological tests such as assimilation of lactose, xylitol, 5-keto-D-gluconate, succinate and citrate as well as growth on media containing 10 % (w/v) NaCl and 5 % (w/v) glucose. In addition, it was established that these strains could utilize up to 10 mM NaCN as sole carbon source on solid media and as sole nitrogen source in liquid media. On the basis of these findings, it is suggested that the three strains represent a novel species for which the name *Cryptococcus cyanovorans* sp. nov. is given (type strain CBS 11948^T=NRRL Y-48730^T).

Isolation of yeasts from industrial wastewaters and related habitats, such as contaminated soils, has become the subject of research due to the economic and public impacts that arise from these sites (Boening & Chew, 1999; Kjeldsen, 1999). Industrial processes such as gold and silver extraction through cyanidation contribute to the production of a number of toxic cyanide compounds that affect living organisms, causing serious environmental and health concerns (Atkinson *et al.*, 1998; Rachinger *et al.*, 2002; Pham *et al.*, 2007). Previous efforts to combat contamination by cyanides were based on the use of chemicals such as ferrous sulphate (Adams, 1992). Currently, the use of micro-organisms to transform or mineralize toxic pollutants has gained prominence as a relevant substitute to reduce the toxicity of polluting cyanides (Boopathy, 2000).

Bioremediation of cyanides is mainly reported in studies focusing on the use of algae, filamentous fungi and bacteria (Dumestre *et al.*, 1997; Barclay *et al.*, 1998; Akcil, 2003; Ezzi & Lynch, 2005; Gurbuz *et al.*, 2009). Although studies have reported yeast strains that can bioremediate heavy metals, including chromium, copper, zinc and nickel [e.g.

Meyerozyma (Pichia) guilliermondii and *Saccharomyces cerevisiae*] (Machado *et al.*, 2008) as well as organic cyanides such as benzonitrile (*Cryptococcus* sp. UFMSY-28) and phenylacetone nitrile (e.g. *Exophiala oligosperma*) (Rezende *et al.*, 2000; Rustler & Stolz, 2007), only one strain, *Cryptococcus humicola* MCN2, is reported to biodegrade inorganic cyanide compounds (Kwon *et al.*, 2002).

During the study of unidentified yeast isolates deposited in the UNESCO-MIRCEN Biotechnological Yeast Culture Collection housed at the Department of Microbial, Biochemical and Food Biotechnology of the University of the Free State, South Africa, a group of isolates obtained from cyanide-contaminated soil from Sasolburg, South Africa were identified. These strains were all isolated from the same site; however, they were obtained from different soil samples taken from the site. Three of these strains, each isolated from a different sample, were found to belong to a previously undescribed species in the genus *Cryptococcus*. The name *Cryptococcus cyanovorans* sp. nov. is proposed for this novel species.

Isolation of DNA was performed on yeast cells grown for approximately 24 h at 30 °C in yeast malt extract (YM) broth (per litre, 3 g yeast extract, 3 g malt extract, 5 g peptone and 10 g glucose). Isolation of DNA was achieved either by genomic DNA extraction (Labuschagne & Albertyn, 2007) or by heating (10 min at 96 °C) cells from a single colony suspended in 20 µl triple distilled water. PCR was performed for the 26S rDNA gene D1/D2 and ITS

The GenBank/EMBL/DDBJ accession numbers for the 26S rDNA gene D1/D2 domain of strains CBS 11948^T, CBS 11949 and CBS 11950 are JF680899, JF680897 and JF680901, respectively. The GenBank/EMBL/DDBJ accession numbers for the internal transcribed spacer regions of strains CBS 11948^T, CBS 11949 and CBS 11950 are JF680900, JF680898 and JF680902, respectively.

Table 1. Strains identified from cyanide-contaminated soil

UOFS, strains are maintained in the UNESCO-MIRCEN Biotechnology Yeast Culture Collection, University of the Free State.

Species	Strains
Ascomycetous yeasts	
<i>Candida rugosa</i>	UOFS Y-2229
<i>Candida sorbophila</i>	UOFS Y-2372
Basidiomycetous yeasts	
<i>Cryptococcus curvatus</i>	UOFS Y-2254
<i>Cryptococcus curvatus</i>	UOFS Y-2263
<i>Cryptococcus cyanovorans</i> sp. nov.	CBS 11948 ^T (=NRRL Y-48730 ^T)
<i>Cryptococcus cyanovorans</i> sp. nov.	CBS 11949 (=NRRL Y-48728)
<i>Cryptococcus cyanovorans</i> sp. nov.	CBS 11950 (=NRRL Y-48729)
<i>Rhodospiridium toruloides</i>	UOFS Y-2233
<i>Rhodospiridium toruloides</i>	UOFS Y-2257
<i>Rhodospiridium toruloides</i>	UOFS Y-2265
<i>Rhodospiridium toruloides</i>	UOFS Y-2234
<i>Rhodospiridium toruloides</i>	UOFS Y-2373
<i>Rhodospiridium toruloides</i>	UOFS Y-2261
<i>Rhodospiridium toruloides</i>	UOFS Y-2247
<i>Rhodospiridium toruloides</i>	UOFS Y-2255
<i>Rhodospiridium toruloides</i>	UOFS Y-2231
<i>Rhodospiridium toruloides</i>	UOFS Y-2370
<i>Trichosporon montevidense</i>	UOFS Y-2266

regions using fungal primers NL-1 (5'-GCATATCAA-TAAGCGGAGGAAAAG-3') and NL-4 (5'-GGTCCGTGT-TTCAAGACGG-3'), ITS-4 (5'-TCCTCCGCTTATTGATATGS-3') and ITS-5 (5'-GGAAGTAAAAGTCGTAAC-AAGG-3'), respectively (White *et al.*, 1990; Kurtzman & Robnett, 1998). Sequences for both the rDNA genes and ITS regions were obtained with the ABI BigDye Terminator Cycle sequencing kit (Applied Biosystems). Resulting sequences were analysed using Geneious Pro 4.8 (Drummond *et al.*, 2009) and compared with sequences in GenBank using the BLAST search algorithm (<http://www.ncbi.nlm.nih.gov>). Sequences were aligned using CLUSTAL_X version 2.0 (Larkin *et al.*, 2007). Phylogenetic and molecular evolutionary analyses were conducted with MEGA version 4 (Tamura *et al.*, 2007) using the neighbour-joining method with the Kimura two-parameter distance measure. Confidence values were estimated from bootstrap analysis of 1000 replicates.

Morphological and physiological properties of strains were determined according to standard methods (van der Walt & Yarrow, 1984; Yarrow, 1998). Scanning electron microscopy (SEM) was conducted on 3-day-old cultures, incubated at 25 °C on YM agar. The cells were fixed according to van Wyk & Wingfield (1994) and scanning electron micrographs were taken with a JEOL 6400 WIN-SEM. The ability of these novel strains, as well as other strains isolated from cyanide-contaminated soil and *Cryptococcus curvatus* CBS 570^T and *Cryptococcus humicolus* CBS 2042, to grow in the presence of NaCN as sole nitrogen source in liquid media was assessed as follows. Cells from strains were

starved by cultivation in yeast nitrogen base (YNB) without ammonium sulphate and amino acids (per litre 1.7 g YNB and 10 g glucose) following the procedure of Müller *et al.* (2007). After this period, cells were tested for nitrogen source assimilation using minimal medium (containing, per litre, 4.3 g K₂HPO₄, 3.4 g KH₂PO₄ and 0.3 g MgCl₂) containing 10 g l⁻¹ glucose and 10 mM NaCN. Cells were incubated for 120 h at 30 °C. The ability of strains to assimilate NaCN as sole carbon source was assessed using complete YNB medium (6.7 g l⁻¹) containing 10 mM NaCN and incubated at 30 °C for 10 days.

Eighteen previously unidentified strains, isolated from cyanide-contaminated soil and held in the UNESCO-MIRCEN Biotechnological Yeast Culture Collection, University of the Free State, South Africa, were identified during this study (Table 1). The ascomycetous yeasts identified were *Candida rugosa* and *Candida sorbophila* and the basidiomycetous yeasts identified were *Cryptococcus curvatus*, *Rhodospiridium toruloides* and *Trichosporon montevidense*. Three strains were found to represent a previously undescribed species in the genus *Cryptococcus*.

The basidiomycete genus *Cryptococcus* Vuillemin is polyphyletic and comprises more than 50 anamorphic species distributed in the class Tremellomycetes, orders Cystofilobasidiales, Filobasidiales, Tremellales and Trichosporonales (Fell *et al.*, 2000; Scorzetti *et al.*, 2002; Golubev *et al.*, 2006; Boekhout *et al.*, 2011). Because of the polyphyletic nature of species of the genus *Cryptococcus*, proper phylogenetic positioning is often difficult and requires comprehensive and thorough analyses of sequence data (Nakase *et al.*, 1993; Fell *et al.*, 2000). In addition, phylogenetic groups within orders such as the Trichosporonales are frequently redescribed based on the inclusion of previously unknown species that may represent unrecognized subgroups. The latter order is described in literature based on six subgroups, i.e. Cutaneum, Formosensis, Gracile/Brassicaceae, Humicola, Ovoides and Porosum (Takashima *et al.*, 2001; Scorzetti *et al.*, 2002; Middelhoven *et al.*, 2004; Takashima *et al.*, 2009; Pagnocca *et al.*, 2010). The Humicola subgroup was reported to be the only group in the order Trichosporonales comprising only members of the genus *Cryptococcus* (i.e. *C. humicola*, *C. longus*, *C. musci*, *C. ramirezgomezianus* and *C. pseudolongus*) while other members of this genus in this lineage, such as *Cryptococcus curvatus*, *Cryptococcus daszewskae* and *Cryptococcus fragicola*, are poorly supported (Takashima *et al.*, 2001; Okoli *et al.*, 2007; Takashima *et al.*, 2009). The current paper proposes the description of *Cryptococcus cyanovorans* sp. nov. (type strain CBS 11948^T, additional strains CBS 11949 and CBS 11950) which, together with the closely related species *Cryptococcus curvatus*, represent another subgroup (Curvatus), comprising only members of the genus *Cryptococcus* in the order Trichosporonales (Fig. 1).

Fig. 1. Phylogenetic placement of *Cryptococcus cyanovorans* sp. nov. in the Curvatus subgroup using the neighbour-joining method with the Kimura two-parameter distance measure. Analysis was performed based on concatenated sequences of 26S rDNA gene D1/D2 region and the ITS domain. Bootstrap percentage (1000 replicates) of $\geq 50\%$ are shown. Bar, 2% nucleotide sequence divergence. *Cryptococcus amylolentus* was used as the outgroup species in the analysis. Sequences were retrieved from the GenBank/EMBL/DBJ database using the accession numbers indicated on the tree.

Physiological and biochemical studies demonstrated that the three novel *Cryptococcus* strains conformed to the current description of this genus as observed by the utilization of D-glucuronate and myo-inositol as well as positive reactions for Diazonium Blue B salt and urease activity (Fell & Statzell-Tallman, 1998; Fonseca *et al.*, 2011). In addition, these strains were unable to ferment D-glucose. The strains demonstrated consistent differences in their abilities to assimilate certain carbon and nitrogen sources, to grow in the presence of 0.1% cycloheximide, to produce extracellular starch and to liquefy gelatin (Table 2). This, considered together with the fact that each of the strains was obtained from a different soil sample taken from the site, demonstrates the independence of the three strains. Growth studies on cyanide revealed that *Cryptococcus cyanovorans* sp. nov. was able to assimilate up to 10 mM NaCN as sole carbon and nitrogen source. This species is phenotypically distinct from *Cryptococcus curvatus*, as indicated in Table 3. The only other strain in this study that was able to assimilate cyanide, albeit to a lesser extent, was *Rhodospiridium toruloides* UOFS Y-2233.

Table 2. Phenotypic characteristics of the three different strains of *Cryptococcus cyanovorans* sp. nov.

+, Positive; –, negative.

Characteristic	CBS 11948 ^T	CBS 11949	CBS 11950
Carbon source assimilation:			
L-Arabinose	–	+	–
Raffinose	+	–	+
Erythritol	+	–	+
Ribitol	+	+	–
Glucitol	–	+	+
Lactate	+	+	–
Propane-1,2-diol	+	+	–
Butane-2,3-diol	+	–	–
Nitrogen source assimilation:			
Nitrate	+	–	+
Nitrite	+	–	+
L-Lysine	+	+	–
Cadaverine	+	–	+
Creatinine	+	–	–
Imidazole	+	–	+
Growth in 0.01% cycloheximide	–	+	+
Starch formation	+	–	+
Gelatin liquefaction	–	+	–

Latin diagnosis of *Cryptococcus cyanovorans* Motaung, Albertyn, J. L. F. Kock et Pohl sp. nov.

In medio liquid extracto fermenti confecto post dies tres ad 25 °C, cellulae globosae ad cylindricae (1.2–7.2 µm × 1.2–8.8 µm). Cellulae polariter gemmantes, circumglobatae et binae coherentes. In agar extracto fermenti et extracto malti confecto, post dies tres ad 25 °C, cultura glabra, cremea, margine integra aut fimbriata. In agar Dalmau nec pseudohyphae nec hyphae formantur. Non fermentat. D-glucosum, D-galactosum, N-acetylglucosaminum, ribosum, xylosum, L-arabiosum (variabile), sucrosum, maltosum, α,α-trehalosum, methyl α-glucosidum, cellobiosum, salicinum, arbutinum, raffinose (variable), melezitolum, glycerolum, erythritolum (variabile), ribitolum (variabile), glucitolum (variabile), myo-inositolum, gluconatum, D-glucuronatum, lactatum (variabile), malatum, propan-1, 2-diolum (variabile), butan-2,3-diolum (variabile) et cyanidum assimilantur, neque sorbosum, rhamnosum, lactosum, inulinum, amyllum solubile, xylitolum, arabitolum, mannitolum, dulcitolum, 2-keto D-gluconatum, 5-keto D-gluconatum, succinatum, citratum, ethanolum, methanolum, saccharatum, hexadecanum. Nitratum (variabile), nitritum (variabile), ethylaminum, L-lysinum (variabile), cadaverinum (variabile), imidazolium (variabile) et cyanidum assimilantur. Ureum finditur. Vitamina externa non necessaria. Materia amyloidea formatur (variabile). In medio 0.01% cycloheximidum crescit (variabile). Crescit in medio 1% aceticum acidum et 10% NaCl/5% glucosum neque in medio 50% glucosum contente. Gelatinum non liquescit. Crescit ad 37 °C. Typus praeservatus in collectione zymotica Centraalbureau voor Schimmelcultures, Utrecht, Neerlandia (CBS 11948^T) et in collectione zymotica Agricultural Research Services United States Department of Agriculture, Peoria, Illinois (NRRL Y-48730^T).

Table 3. Distinguishing characteristics of *Cryptococcus cyanovorans* sp. nov. and the related species *Cryptococcus curvatus* (taken from Boekhout *et al.*, 2011)

Assimilation	<i>C. cyanovorans</i>	<i>C. curvatus</i>
Carbon source		
Lactose	–	+
Xylitol	–	+
5-Keto D-gluconate	–	+
Succinate	–	+
Citrate	–	+
Additional tests		
10% NaCl/5% D-Glucose	+	–

Table 4. Assimilation of carbon and nitrogen sources by *Cryptococcus cyanovorans* sp. nov.

+, Positive; –, negative; v, variable.

Assimilation	<i>Cryptococcus cyanovorans</i> sp. nov.
Carbon source	
D-Glucose	+
D-Galactose	+
Sorbose	–
N-Acetylglucosamine	+
Ribose	+
Xylose	+
L-Arabinose	v
Rhamnose	–
Sucrose	+
Maltose	+
α,α -Trehalose	+
Methyl α -glucoside	+
Cellobiose	+
Salicin	+
Arbutin	+
Lactose	–
Raffinose	v
Melezitose	+
Inulin	–
Starch	–
Glycerol	+
Erythritol	v
Ribitol	v
Xylitol	–
Arabitol	–
Glucitol	v
Mannitol	–
Dulcitol	–
myo-Inositol	+
2-Keto D-gluconate	–
5-Keto D-gluconate	–
Gluconate	+
D-Glucuronate	+
Lactate	v
Succinate	–
Citrate	–
Malate	+
Ethanol	–
Methanol	–
Propane-1,2-diol	v
Butane-2,3-diol	v
Saccharate	–
Hexadecane	–
Cyanide	+
Nitrogen source	
Nitrate	v
Nitrite	v
Ethylamine	+
L-Lysine	v
Cadaverine	v
Creatinine	v
Imidazole	v
Cyanide	+

Fig. 2. Globose to elongate budding cells of *Cryptococcus cyanovorans* sp. nov. after 3 days at 25 °C on YM agar. Bar, 5 µm.

Description of *Cryptococcus cyanovorans* Motaung, Albertyn, J. L. F. Kock & Pohl sp. nov.

Cryptococcus cyanovorans (cy.a.no.vo'rans. N.L. adj. *cyano-* referring to the ability of this strain to assimilate cyanide).

In yeast extract peptone medium after 3 days at 25 °C, cells are globose to elongate (1.2–7.2 µm × 1.2–8.8 µm). Mono- and bipolar budding is observed and cells occur in clusters or pairs (Fig. 2). In yeast malt extract (YM) agar after 3 days at 25 °C, colonies are smooth, cream-coloured with entire or fringed margins. In Dalmau plate cultures on cornmeal agar, pseudo or true mycelia are not formed. No fermentation of carbon compounds is observed. The carbon and nitrogen source assimilation profiles are given in Table 4. Urea is hydrolysed and vitamins are not required for growth. Starch production and growth in the presence of 0.01 % cycloheximide is variable. Growth is observed on 1 % acetic acid and 10 % NaCl plus 5 % glucose, but not on 50 % glucose. Gelatin is not liquefied. Growth is observed at 37 °C.

The type strain, CBS 11948^T (=NRRL Y-48730^T), as well as two additional strains, CBS 11949 (=NRRL Y-48728) and CBS 11950 (=NRRL Y-48729), are deposited in the yeast culture collection of the Centraalbureau voor Schimmelfcultures, Utrecht, The Netherlands, with copies at the Agricultural Research Service Culture Collection, United States Department of Agriculture, Peoria, Illinois, USA.

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References

- Adams, A. D. (1992). The removal of cyanide from aqueous solution by the use of ferrous sulphate. *J S Afr Inst Min Metall* **92**, 17–26.
- Akcil, A. (2003). Destruction of cyanide in gold mill effluents: biological versus chemical treatments. *Biotechnol Adv* **21**, 501–511.
- Atkinson, B. W., Bux, F. & Kasan, H. C. (1998). Considerations for application of biosorption technology to remediate metal-contaminated industrial effluents. *Water SA* **24**, 129–136.
- Barclay, M., Tett, V. A. & Knowles, C. J. (1998). Metabolism and enzymology of cyanide/metalocyanide biodegradation by *Fusarium solani* under neutral and acidic conditions. *Enzyme Microb Technol* **23**, 321–330.
- Boekhout, T., Fonseca, Á., Sampaio, J. P., Bandoni, R. J., Fell, J. W. & Kwon-Chung, K. J. (2011). Discussion of teleomorphic and anamorphic basidiomycetous yeasts. In *The Yeasts, a Taxonomic Study*, 5th edn, pp. 1339–1372. Edited by C. P. Kurtzman, J. W. Fell & T. Boekhout. Amsterdam: Elsevier.
- Boening, D. W. & Chew, C. M. (1999). A critical review: general toxicity and environmental fate of three aqueous cyanide ions and associated ligands. *Water Air Soil Pollut* **109**, 67–79.
- Boopathy, R. (2000). Factors limiting bioremediation technologies. *Bioresour Technol* **74**, 63–67.
- Drummond, A. J., Ashton, B., Cheung, M., Heled, J., Kears, M., Moir, R., Stones-Havas, S., Thierer, T. & Wilson, A. (2009). Geneious v4.7, available from <http://www.geneious.com>
- Dumestre, A., Chone, T., Portal, J., Gerard, M. & Berthelin, J. (1997). Cyanide degradation under alkaline conditions by a strain of *Fusarium solani* isolated from contaminated soils. *Appl Environ Microbiol* **63**, 2729–2734.
- Ezzi, M. I. & Lynch, J. M. (2005). Biodegradation of cyanide by *Trichoderma* spp. and *Fusarium* spp. *Enzyme Microb Technol* **36**, 849–854.
- Fell, J. W. & Statzell-Tallman, A. (1998). *Cryptococcus* Vuillemin. In *The Yeasts, a Taxonomic Study*, 4th edn, pp. 742–767. Edited by C. P. Kurtzman & J. W. Fell. Amsterdam: Elsevier.
- Fell, J. W., Boekhout, T., Fonseca, Á., 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.
- Fonseca, Á., Boekhout, T. & Fell, J. W. (2011). *Cryptococcus* Vuillemin. In *The Yeasts, a Taxonomic Study*, 5th edn, pp. 1661–1737. Edited by C. P. Kurtzman, J. W. Fell & T. Boekhout. Amsterdam: Elsevier.
- Golubev, W. I., Sampaio, J. P. & Golubeva, E. W. (2006). *Cryptococcus stepposus*, a new filobasidiaceous yeast species found in the Prioksko-terrasny biosphere reserve in Russia. *Mycol Res* **110**, 957–961.
- Gurbuz, F., Ciftci, H. & Akcil, A. (2009). Biodegradation of cyanide containing effluents by *Scenedesmus obliquus*. *J Hazard Mater* **162**, 74–79.
- Kjeldsen, P. (1999). Behaviour of cyanides in soil and groundwater: a review. *Water Air Soil Pollut* **115**, 279–308.
- Kurtzman, C. P. & Robnett, C. J. (1998). Identification and phylogeny of ascomycetous yeasts from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences. *Antonie van Leeuwenhoek* **73**, 331–371.
- Kwon, H. K., Woo, S. H. & Park, J. M. (2002). Degradation of tetracyanonickelate (II) by *Cryptococcus humicola* MCN2. *FEMS Microbiol Lett* **214**, 211–216.
- Labuschagne, M. & Albertyn, J. (2007). Cloning of an epoxide hydrolase-encoding gene from *Rhodotorula mucilaginosa* and functional expression in *Yarrowia lipolytica*. *Yeast* **24**, 69–78.
- Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., McGettigan, P. A., McWilliam, H., Valentin, F., Wallace, I. M., Wilm, A. & other authors (2007). CLUSTAL W and CLUSTAL_X version 2.0. *Bioinformatics* **23**, 2947–2948.
- Machado, M. D., Santos, M. S. F., Gouveia, C., Soares, H. M. V. M. & Soares, E. V. (2008). Removal of heavy metals using a brewer's yeast strain of *Saccharomyces cerevisiae*: the flocculation as a separation process. *Bioresour Technol* **99**, 2107–2115.
- Middelhoven, W. J., Scorzetti, G. & Fell, J. W. (2004). 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*. *Int J Syst Evol Microbiol* **54**, 975–986.
- Müller, W. J., Albertyn, J. & Smit, M. S. (2007). Cycloheximide resistance in the Lipomycetaceae revisited. *Can J Microbiol* **53**, 509–513.
- Nakase, T., Takematsu, A. & Yamada, Y. (1993). Molecular approaches to the taxonomy of ballistosporeous yeasts based on the analysis of the partial nucleotide sequences of 18S ribosomal ribonucleic acids. *J Gen Appl Microbiol* **39**, 107–134.
- Okoli, I., Oyeka, C. A., Kwon-Chung, K. J., Theelen, B., Robert, V., Groenewald, J. Z., McFadden, D. C., Casadevall, A. & Boekhout, T. (2007). *Cryptotrichosporon anacardii* gen. nov., sp. nov., a new trichosporonoid capsulate basidiomycetous yeast from Nigeria that is able to form melanin on niger seed agar. *FEMS Yeast Res* **7**, 339–350.
- Pagnocca, F. C., Legaspe, M. F., Rodrigues, A., Ruivo, C. C., Nagamoto, N. S., Bacci, M., Jr & Forti, L. C. (2010). Yeasts isolated from a fungus-growing ant nest, including the description of *Trichosporon chiarellii* sp. nov., an anamorphic basidiomycetous yeast. *Int J Syst Evol Microbiol* **60**, 1454–1459.
- Pham, J. C., Huang, D. T., McGeorge, F. T. & Rivers, E. P. (2007). Clarification of cyanide's effect on oxygen transport characteristics in a canine model. *Emerg Med J* **24**, 152–156.
- Rachinger, J., Fellner, F. A., Stieglbauer, K. & Trenkler, J. (2002). MR changes after acute cyanide intoxication. *AJNR Am J Neuroradiol* **23**, 1398–1401.
- Rezende, R. P., Teixeira Dias, J. C., Ferraz, V. & Linardi, V. R. (2000). Metabolism of benzonitrile by *Cryptococcus* sp. UFMG-Y28. *J Basic Microbiol* **40**, 389–392.
- Rustler, S. & Stolz, A. (2007). Isolation and characterization of a nitrile hydrolysing acidotolerant black yeast-*Exophiala oligosperma* R1. *Appl Microbiol Biotechnol* **75**, 899–908.
- Scorzetti, G., Fell, J. W., Fonseca, Á. & 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.
- Takashima, M., Sugita, T., Shinoda, T. & Nakase, T. (2001). Reclassification of the *Cryptococcus humicola* complex. *Int J Syst Evol Microbiol* **51**, 2199–2210.
- Takashima, M., Sugita, T., Toriumi, Y. & Nakase, T. (2009). *Cryptococcus tepidarius* sp. nov., a thermotolerant yeast species isolated from a stream from a hot-spring area in Japan. *Int J Syst Evol Microbiol* **59**, 181–185.
- Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007). MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol Biol Evol* **24**, 1596–1599.
- van der Walt, J. P. & Yarrow, D. (1984). Methods for isolation, maintenance, classification and identification of yeasts. In *The Yeasts*,

a Taxonomic Study, 3rd edn, pp. 45–101. Edited by N. J. W. Kreger-van Rij. Amsterdam: Elsevier.

van Wyk, P. W. J. & Wingfield, M. J. (1994). Ultrastructure of ascus arrangement and ascospore development in *Ophiostoma seticolle*. *Mycologia* **86**, 607–614.

White, T. J., Bruns, T., Lee, S. & Taylor, J. W. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In

PCR Protocols: A Guide to Methods and Applications, pp. 315–322. Edited by M. A. Innis, D. H. Gelfand, J. J. Sninsky & T. J. White. San Diego, CA: Academic Press.

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.