The ascomycetous yeast genus Metschnikowia is defined by the presence of multilateral budding of vegetative cells and by the production of one or two needle-shaped ascospores in elongated asci (Miller & Phaff, 1998). Species isolated from terrestrial habitats are typically associated with flowers or fruits and transmitted to new niches by insects; species isolated from aquatic habitats are often parasitic in invertebrates but can also be isolated as free-living forms in water (Miller & Phaff, 1998). The extent of rRNA sequence diversity among Metschnikowia species is surprisingly high considering the relative phenotypic homogeneity of these taxa (Mendonca-Hagler et al., 1993). It has also been suggested that the genus has numerous missing taxa because inclusion of several undescribed species has considerably shortened the genus has numerous missing taxa because inclusion of several undescribed species has considerably shortened the extent of rRNA sequence diversity among Metschnikowia species is surprisingly high considering the relative phenotypic homogeneity of these taxa (Mendonca-Hagler et al., 1993). It has also been suggested that the genus has numerous missing taxa because inclusion of several undescribed species has considerably shortened some terminal branches (Kurtzman & Robnett, 1998).

In the course of an isolation programme of yeasts from various natural sources in Korea, five strains of yeast were isolated from Ipomoea sp. and Lilium sp. from one location. Determination of the nucleotide sequence of the D1/D2 domain of the 26S rDNA of the strains and comparison with a database of sequences from all currently recognized yeasts confirmed that five strains represent novel species. Two strains were included in the Sporidiobolus clade (Fell et al., 2000) and three strains were included in the Metschnikowia clade (Kurtzman & Robnett, 1998). Among them, two strains had similarity physiological, biochemical and molecular characteristics. These two strains (SG99-34T and SG99-25) are described here as Metschnikowia koreensis sp. nov.

The morphological and physiological characteristics of strains SG99-34T and SG99-25 were determined by conventional techniques described by Yarrow (1998). The utilization of carbon sources was examined at 25 °C on a rotary shaker (120 r.p.m.) at 7 d intervals for 21 d. Carbon sources that were utilized only after more than 7 d were scored as a delayed reaction and weakly positive assimilation during 3 weeks was scored as weak. The utilization of nitrogen sources was examined by the auxanographic technique for 7 d. Urease activity was tested in Christensen’s urea agar (Christensen, 1946). Determination of the coenzyme Q system was carried out as described by Yamada (1998) with an HPLC equipped with a Spherisorb S5 ODS2 column (Waters). The DNA base composition (G+C mol%) was determined by examining Tm. As a control, a DNA preparation from Escherichia coli KCTC 2443 (G+C content = 51-0 mol%) was included. DNA–DNA hybridization was performed using the DIG High Prime DNA Labelling and Detection Starter kit II (Roche) and a Bio-Dot SF slot blotting apparatus (Bio-Rad) following the manufacturers’ instructions.

For transmission electron microscopy, material was fixed in a 2-5% paraformaldehyde-glutaraldehyde fixative mixture buffered with 0-1 M phosphate (pH 7-2) for 2 h at 4 °C. The sample was washed three times with 0-1 M phosphate (pH 7-2), post-fixed in 1% osmium tetroxide in the same buffer for 1 h, dehydrated in graded ethanol and propylene oxide, and then embedded in Epon-812. Thin sections (80 nm), made using the Reichert-Jung ULTRACUT E (Leica) ultramicrotome, were stained in a saturated uranyl acetate solution in distilled water (2 h at 25 °C) followed by lead citrate (15 min at 25 °C). A CM 20 (Philips) electron microscope was used for analysis.

The D1/D2 domain of nuclear 26S rDNA was amplified and sequenced using the primer pair no. 4 (ACCCG CTGAA YTTAA GCATA T) and no. 11

Keywords: yeast, Metschnikowia, 26S rDNA, Metschnikowia koreensis sp. nov.
The amplified 26S rDNAs were purified using a Wizard PCR prep (Promega). The nucleotide sequences were determined with BigDye terminator cycle sequencing kits (PE Applied Biosystems) following the manufacturer’s instructions. The gel electrophoresis and data collection were performed on an ABI Prism 310 Genetic Analyzer (PE Applied Biosystems). The sequences were proofread, edited and merged into composite sequences using the PHYLIT program version 3.0 (Chun, 1995). The 26S rDNA D1/D2 sequences of the strains were aligned with other 26S rDNA sequences from the genus Metschnikowia (Kurtzman & Robnett, 1998; Lachance et al., 1998) on the basis of similarity of the primary and secondary structures using the PHYLIT program. Tree topology was reconstructed with the Kimura’s 2-parameter distance model (Kimura, 1980) and the neighbour-joining method (Saitou & Nei, 1987) using the PHYLIP 3.05 package (Felsenstein, 1993). Saccharomyces cerevisiae was used as the outgroup. Confidence levels for the individual branches of the resulting tree were assessed by bootstrap analysis (Felsenstein, 1985) in which 1000 bootstrapped trees were generated from the resampled data. The resultant phylogenetic trees were visualized using the TREEVIEW program (Page, 1996).

The phylogenetic placement of strain SG99-34T was determined by analysis of the D1/D2 domain sequences of 26S rDNA from all currently recognized ascomycetous yeast species, as reported in the studies of Kurtzman & Robnett (1998) and Lachance et al. (1998). Strain SG99-34T clustered with the core group of the genus Metschnikowia including Metschnikowia bicuspidata, the type species of the genus, and was closely related to M. reukaufii (Fig. 3). Morphological characteristics of strain SG99-34T also demonstrated that it should be assigned to the genus Metschnikowia. It reproduced by multilateral budding and produced ellipsoidopedunculate asci containing two acerose ascospores.

It has been proposed that yeast strains showing greater than 1% nucleotide substitution in the D1/D2 domain of 26S rDNA are likely to belong to different species (Kurtzman & Robnett, 1998). The sequence similarity of the D1/D2 domain between strain SG99-34T and M. reukaufii NRRL Y-7112T was 93.7%, which strongly implies that strain SG99-34T represents a unique species. Strain SG99-34T can be differentiated phenotypically from M. reukaufii by its ability to assimilate d-glucosamine, lack of growth on hexadecane, growth in 16% NaCl/5% glucose, production of urease and growth at 35°C (Miller & Paff, 1998; Pitt & Miller, 1968). Urea hydrolysis is rarely observed in ascomycetous yeast, apart from in Schizosaccharomyces pombe, Yarrowia lipolytica and Lipomyces sp. (Yarrow, 1998). Therefore, it can be used as a unique characteristic to distinguish M. koreensis from other Metschnikowia species. On the basis of molecular and physiological characteristics, it is fair to describe strain SG99-34T as a novel species in the genus Metschnikowia.

Strain SG99–25 differed in the following characteristics from SG99-34T. The sequence difference of the D1/D2 domain between the two strains was 1 out of 532 sites. It produced ascii in 1:29 diluted V8 agar rather than 1:9 diluted V8 agar and formation of ascii was not frequent. The major ubiquinone of both strains was Q9, but SG99-25 contained considerable amounts (Q9:Q8 = 4:1) of the Q8 ubiquinone system. The assimilation of d-galactose and ethanol was negative and weak, respectively. Because of the considerable differences in physiological and biochemical characteristics between the two strains, chromosomal DNA similarity was analysed by DNA–DNA hybridization to test conspecificity. The result of 91% similarity indicated that the two strains are conspecific.

Latin diagnosis of Metschnikowia koreensis Hong, Chun, Oh et Bae sp. nov.


Description of Metschnikowia koreensis sp. nov.

Metschnikowia koreensis (ko.re.én’sis. L. nom. fem. adj. koreensis of Korea, referring to the country where the yeast was isolated).
Metschnikowia koreensis sp. nov.

In 5% malt extract broth after 3 d at 25 °C, the cells are ellipsoidal to short cylindrical shapes (3–6 × 4–7 µm). They mainly occur singly or in short chains. Vegetative reproduction proceeds by multilateral budding (Fig. 1, top). A sediment forms after 4 weeks. A pellicle is absent. On 10% malt extract agar after 3 d at 25 °C, the colonies are white to cream-coloured and butyrous. Two morphotypes become apparent after repeated subculturing: one has a somewhat rough and dull surface and the other is smooth and slightly glistening. The former consists mainly of actively reproducing vegetative cells and the latter consists mainly of refractile subglobose chlamydospores (6–8 × 7–9 µm; Fig. 1, bottom). The margins are usually smooth, but fringed in some cases. In Dalmau plate cultures on cornmeal agar after 10 d at 25 °C, two types of cells are observed in one colony: one is subglobose and glistening under the microscope and the other has cylindrical shape and dull texture. Pseudomycelium is rudimentary or absent. Sporulation of strain SG99-34T is observed on dilute (1:9) V8 agar after 2 weeks incubation at 20 °C. Ellipsoido-pedunculate asci (4–5 × 14–20 µm) containing two ascospores arise from chlamydospores (Fig. 2). The ascospores are acerose (13–19 × 1 µm). Asci do not lyse at maturity. Strain SG99-25 produces ascii on dilute (1:29) V8 agar. D-Glucose is fermented; D-
galactose, sucrose, maltose, lactose, raffinose and trehalose are not fermented. Assimilation of carbon compounds is as follows: d-glucose, +; d-galactose, + (latently); l-sorbitose, +; sucrose, +; maltose, +; cellulbiose, +; trehalose, +; lactose, −; melibiose, −; raffinose, −; melezitose, +; inulin, −; soluble starch, −; d-xyllose, +; l-arabinose, −; d-arabinose, −; d-ribose, + (latently); l-rhamnose, −; d-glucosamine, +; N-acetyl-d-glucosamine, +; methanol, −; ethanol, +; glycerol, +; erythritol, −; ribitol, +; galactitol, −; d-mannitol, +; d-glucitol, +; methyl a-D-glucoside, +; salicin, +; d-gluconate, +; DL-lactate, −; succinate +; citrate, −; inositol, −; hexadecane, −; and glucuronate, −. Assimilation of nitrogen compounds is as follows: nitrate, −; nitrite, −; ethylamine, +; cadaverine, +; L-lysine, +; d-glucosamine, −; creatine, −; and creatinine, −. Growth or response in other tests: 10% NaCl/5% glucose, +; 16% NaCl/5% glucose, +; 0·01% cycloheximide, −; 0·1% cycloheximide, −; 1% acetic acid, −; starch formation, −; urease, +; growth at 35 °C, +; growth at 37 °C, −; vitamin-free medium, −; Diazonium Blue B, −. The major ubiquinone system of strain SG99-34t is Q9. However, Q8 is also present in small proportions (39.1). The amount of Q8 is higher in strain SG99-25 than in strain SG99-34t (4.1). The G + C molar ratio is 41.2 ± 0.8 mol % (mean of four determinations). The closest phylogenetic relative of SG99-34t based on the D1/D2 domain sequences of 26S rDNA is Metschnikowia reukaufii NRRL Y-7112T (Fig. 3). The similarity of the two sequences is 93.7%. The sequence difference between strains SG99-34t and SG99-25 is 1 out of 532 sites. The type strain SG99-34t (=KCTC 7998T = CBS 8854T) and strain SG99-25 (=KCTC 7828) were isolated in August 1999 from flowers of Lilium sp. and Ipomoea sp., respectively, in Kyungsang province, Korea. Strain SG99-25 differs from the type strain in the following assimilation characteristics: it does not assimilate d-galactose and weakly assimilates ethanol.

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**References**


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