

Full Paper

***Candida phyllophila* sp. nov. and *Candida vitiphila* sp. nov., two novel yeast species from grape phylloplane in Thailand**

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Three strains (K59^T, K60 and K70^T) representing two novel yeast species were isolated from the external surface of leaves of different wine grape (*Vitis vinifera*) plants, which were collected from the Kanchanaburi Research Station (N14°07'15.1" E099°19'05.6"), Wang Dong Sub-district, Mueang District, Kanchanaburi Province, Thailand, by an enrichment technique. The sequences of the D1/D2 domain of the large subunit (LSU) rRNA gene of two strains (K59^T and K60) were identical and differed from that of strain K70^T. In terms of pairwise sequence similarity of the D1/D2 domain, the closest species to the three strains was *Candida asparagi* but with 2.3% nucleotide substitutions for strains K59^T and K60, and 2.1% nucleotide substitutions for strain K70^T. On the basis of morphological, biochemical, physiological and chemotaxonomic characteristics and the sequence analysis of the D1/D2 domain of the large subunit (LSU) rRNA gene, the three strains were assigned to be two novel *Candida* species. Two strains (K59^T and K60) were assigned as *Candida phyllophila* sp. nov. (type strain K59^T=BCC 42662^T=NBRC 107776^T=CBS 12671^T). *Candida vitiphila* sp. nov. is proposed for strain K70^T (=BCC 42663^T=NBRC 107777^T=CBS 12672^T).

Key Works—*Candida phyllophila* sp. nov.; *Candida vitiphila* sp. nov.; phylloplane; Thailand

Introduction

The genus *Metschnikowia* is a sister genus to *Clavispora* (Lachance, 2011a, b). Twenty-six *Candida* species have been shown to be in the *Metschnikowia* clade; however, a few *Candida* species in this clade, namely *Candida asparagi*, *Candida fructus*, *Candida musae* and *Candida oregonensis*, form a subclade with the two *Clavispora* species *Clavispora lusitaniae* and *Clavispora opuntiae*, which is separated from

Metschnikowia agaves (Lachance et al., 2011). *C. asparagi* was found to cluster with *C. fructus* and *Cl. lusitaniae* with 7.4% bootstrap support (Lu et al., 2004). Flowers, fruits and other plant tissues, and their associated insects have been found to be the principal habitats of yeasts in the *Metschnikowia* clade (Lachance, 2011b).

The external surface of plant leaves, which is usually referred to as the phylloplane, has been recognized as an important habitat for epiphytic microorganisms (Fonseca and Inacio, 2006; Phaff and Starmer, 1987). Bacteria are the most abundant phylloplane microorganisms while yeasts and yeast-like fungi are also active phylloplane colonizers (Andrews and Harris, 2000). The phylloplanes of diverse plants in temperate and tropical regions have been reported to be colonized

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by the members of both basidiomycetous and ascomycetous yeasts (Fonseca and Inacio, 2006; Glushakova and Chernov, 2010; Glushakova et al., 2007; Inácio et al., 2004, 2005; Landell et al., 2010; Nakase et al., 2001; Slavikova et al., 2009). Basidiomycetous species belonging to the genera *Cryptococcus*, *Rhodotorula*, *Sporobolomyces* and *Trichosporon* have been found to be the most common phylloplane yeasts (de Azeredo et al., 1998; Glushakova and Chernov, 2010; Slavikova et al., 2009); however, in recent years, more ascomycete yeast species have been detected on the phylloplane, such as *Debaryomyces hansenii*, *Hanseniaspora uvarum*, *Kazachstania barnettii*, *Metschnikowia pulcherrima*, *Metschnikowia saccharicola*, *Metschnikowia lophuriensis*, *Pichia membranifaciens*, *Saccharomyces cerevisiae* and various species of *Candida* including *C. aechmeae*, *C. chumphonensis*, *C. maesa*, *C. matranensis*, *C. stauntonica* and *C. vriesiae* (Chang et al., 2012; Glushakova and Chernov, 2010; Kaewwichian et al., 2012; Koowadjanakul et al., 2011; Landell et al., 2010; Slavikova et al., 2009).

During an investigation of phylloplane yeasts of wine grape, table grape and other plant species at the Kanchanaburi Research Station, Kanchanaburi Province, Thailand, three yeast strains obtained from the phylloplane of three wine grapes were found to represent two novel *Candida* species. In this present paper, two strains (K59^T and K60) are described as *Candida phyllophila* sp. nov. and one strain (K70^T) is described as *Candida vitiphila* sp. nov.

Materials and Methods

Yeast isolation. Green and undamaged leaves of grapes were collected from fields on the Kanchanaburi Research Station, Agro-Ecological System Research and Development Institute (N14°07'15.1" E099°19'05.6") of Kasetsart University located in Wang Dong Sub-district, Mueang District, Kanchanaburi Province, Thailand. In total 54 grape leaf samples were collected with 8, 24 and 22 samples collected on 4 February 2009, 2 July 2009 and 2 September 2009, respectively. Yeasts were isolated from the surface of plant leaves by an enrichment technique using yeast extract malt extract (YM) broth (0.3% yeast extract, 0.3% malt extract, 0.5% peptone and 1% glucose) supplemented with 0.025% sodium propionate and 0.02% chloramphenicol (Limtong et al., 2007). Three grams of cut leaves were aseptically placed in 250 ml Erlenmeyer flasks con-

taining 50 ml enrichment broth and incubated on a rotary shaker at room temperature (27±3°C) for 2 days. A loopful of the enriched culture was streaked on YM agar supplemented with 0.025% sodium propionate and 0.02% chloramphenicol and incubated at room temperature until yeast colonies appeared. Yeast colonies of different morphologies were picked and purified by cross streaking on YM agar. Purified yeast strains were suspended in YM broth supplemented with 10% glycerol and maintained at -80°C. One-hundred and one yeast strains were obtained, among these being strains K59 and K60, and K70, which represented two novel species and had been individually isolated from the surface of leaves of three wine grapes (*Vitis vinifera*, Family Vitaceae).

DNA sequencing and phylogenetic analysis. The sequences of the D1/D2 domain of the LSU rRNA gene and the ITS region were determined from PCR products amplified from genomic DNA. Methods for DNA extraction and amplification of the D1/D2 domain of the LSU rRNA gene have been described previously (Limtong et al., 2007). The ITS region was amplified with primers ITS1 and ITS4, following the method of White et al. (1990). The PCR products were checked by agarose gel electrophoresis and purified by using the QIA quick purification kit (Qiagen, Germany). The purified products were sequenced commercially by Macrogen, Inc. (Korea) using the primers NL1 and NL4 for the D1/D2 domain of the LSU rRNA gene and the primers ITS1 and ITS4 for the ITS region. The sequences were compared pairwise using a BLAST search (Altschul et al., 1997) and were aligned with the sequences of related species retrieved from GenBank using the multiple alignment program CLUSTAL_X version 1.81 (Thompson et al., 1997). A phylogenetic tree was constructed from the evolutionary distance data with Kimura's two-parameter correction (Kimura, 1980) using the neighbor-joining method (Saitou and Nei, 1987) with the MEGA software version 5.0 (Tamura et al., 2011). The confidence levels of the clades were estimated from bootstrap analysis using 1,000 replicates (Felsenstein, 1985).

Examination of taxonomic characteristics. The strains were characterized morphologically, biochemically and physiologically according to the standard methods described by Yarrow (1998). Mycelium formation was investigated on cornmeal agar in slide culture at 25°C for up to 7 days. Ascospore formation was investigated on 5% malt extract agar, Fowell's acetate agar, Gorod-

kowa agar and corn meal at 15 and 25°C for up to 4 weeks. Carbon assimilation tests were conducted in liquid medium as described by Yarrow (1998). Assimilation of nitrogen compounds was examined on solid media with starved inocula following the method of Nakase and Suzuki (1986). Growth at various temperatures was determined by cultivation in YM broth. Ubiquinones were extracted from cells cultivated in 500 ml Erlenmeyer flasks containing 250 ml of yeast extract peptone dextrose (YPD) broth (1% yeast extract, 2% peptone and 2% dextrose) on a rotary shaker at 28°C for 24–48 h and purified according to the method described by Yamada and Kondo (1973) and Kuraishi et al. (1985). Isoprenologues were identified by HPLC as described previously (Limtong et al., 2007).

Results and Discussion

Novel species delineation and identification

The analysis of the D1/D2 domain of the LSU rRNA gene sequence revealed that the two strains (K59^T and K60) showed identical sequences and differed from strain K70^T by 3.0% nucleotide substitutions (16 nucleotide substitutions and 4 gaps out of 529 nt). The D1/D2 domains of the LSU rRNA gene sequences of the three strains were closest to *Candida* sp. BG02-7-21-004E-1-1 and *Candida asparagi*. Two strains (K59^T and K60) differed from *Candida* sp. BG02-7-21-004E-1-1 by 2.1% nucleotide substitutions (11 nucleotide substitutions and 2 gaps out of 519 nt) and from *C. asparagi* by 2.3% nucleotide substitutions (12 nucleotide substitutions and 3 gaps out of 529 nt). Strain K70^T differed from *Candida* sp. BG02-7-21-004E-1-1 by 1.5% nucleotide substitutions (8 nucleotide substitutions and 1 gap out of 519 nt) and from *C. asparagi* by 1.7% nucleotide substitutions (9 nucleotide substitutions and 3 gaps out of 529 nt). The nucleotide sequences of the ITS regions of the three strains (K59^T, K60 and K70^T) were also analyzed, but only the sequence of strain K70^T was obtained and deposited as AB736148. The ITS direct sequencing failed for strains K59^T and K60 because the chromatograms always showed overlapped peaks in nearly all positions of DNA strands, although a single band of PCR product was detected, showing that these strains might have heterogenous ITS rRNA gene copies (Egli and Henick-Kling, 2001; Saksinchai et al., 2012). In the ITS region, the sequences of strain K70^T exhibited 6.6% nucleotide substitutions (23 nucleotide substitutions and 4 gaps

out of 349 nt) from its closest described species, *C. asparagi*. The phylogenetic tree in Fig. 1 and based on the sequences of the D1/D2 domain of the LSU rRNA gene further demonstrates that the two strains (K59^T and K60) were at the same position and connected to *Candida* sp. BG02-7-21-004E-1-1. The strain K70^T was connected to those three strains mentioned previously (K59^T, K60 and *Candida* sp. BG02-7-21-004E-1-1) and grouped with *Candida asparagi*, *C. fructus* and *C. musae*. The two novel species were placed in a subclade that contained *Cl. lusitaniae*, *Cl. opuntiae*, *Clavispora reshetovae* and the other *Candida* species that was separated from the subclades that contained the *Metschnikowia* species.

Cells of the three strains (K59^T, K60 and K70^T) were subglobose and ovoid to ellipsoidal (Fig. 2) and proliferated by multilateral budding. Neither pseudohyphae nor true hyphae were formed by any of the three strains. Ascospores were not formed by individual strains or strains paired on 5% malt extract agar, Fowell's acetate agar, Gorodkova agar or cornmeal agar after 4 weeks at 15 and 25°C.

On the basis of morphological, biochemical, physiological and chemotaxonomic characteristics and the sequence analysis of the D1/D2 domain of the LSU rRNA gene, we concluded that the three strains represent two novel *Candida* species. The name *Candida phyllophila* sp. nov. (MB 801036) is proposed for strains K59^T and K60 and *Candida vitiphila* sp. nov. (MB 801037) is assigned to strain K70^T.

The two novel species, *C. phyllophila* sp. nov. and *C. vitiphila* sp. nov. can be separated from each other only on the basis of the sequences of the D1/D2 domain of the LSU rRNA gene while their phenotypic characteristic are the same. They can be distinguished from their closest described species *C. asparagi* not only on the basis of the sequences of the D1/D2 domain of the LSU rRNA gene but also by a few phenotypic characteristics including: assimilation of methyl-D-glucoside, citrate and ethanol by the two novel species compared with delayed assimilation, and weak and delayed assimilation, respectively, by *C. asparagi*; by non assimilation of D-arabinose by the two novel species but weak assimilation by *C. asparagi*; and the ability to grow at 35°C of the two novel species but inability to grow at this temperature of *C. asparagi*.

The principal habitats of members of the *Metschnikowia* clade are flowers, fruits and other plant tissues, and their associated insects (Lachance, 2011b). In the

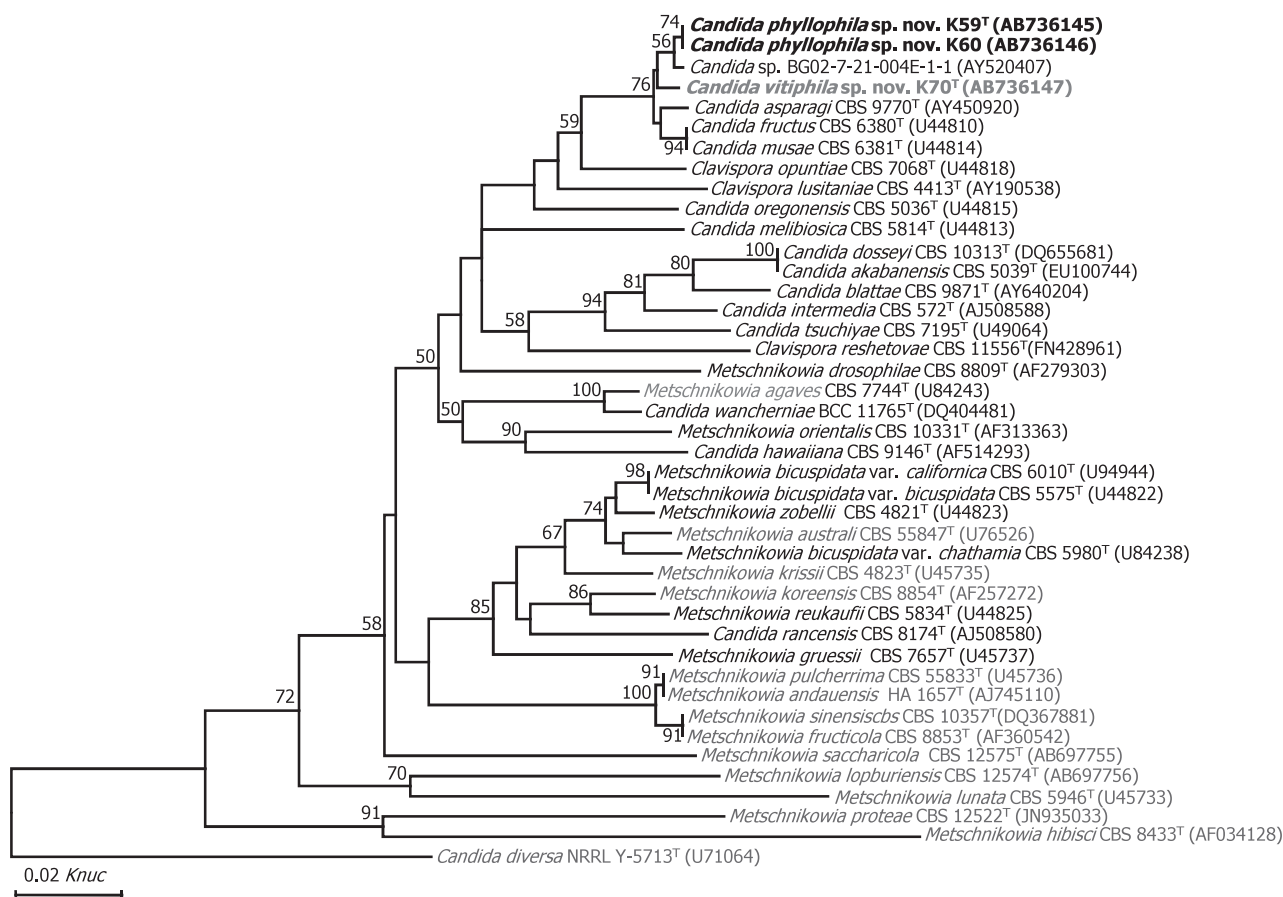


Fig. 1. Phylogenetic tree based on the sequences of the D1/D2 domain of the LSU rRNA gene, showing positions of *Candida phyllophila* sp. nov. (K59^T and K60) and *Candida vitiphila* sp. nov. (K70^T), with respect to closely related species.

The phylogenetic tree was constructed from evolutionary distance data with Kimura's two-parameter correction, using the neighbor-joining method by MEGA software, version 5.0. The numbers at nodes indicate the percentages of bootstrap sampling, derived from 1,000 replicates. The numbers in parentheses are GenBank accession numbers. *Candida diversa* was the out-group species in the analysis.

present investigation, two novel *Candida* species, *C. phyllophila* and *C. vitiphila*, isolated from grape phylloplane were proposed. The limited number of strains derived in this study, although 54 samples of grape leaf were used for yeast isolation, might indicate that yeast in this clade is not commonly found in phylloplane and the occurrence in grape phylloplane may be a consequence of these yeasts being carried to the grape phylloplane by visiting insects. However, the phylloplane appears to be interesting as a source for further investigations of yeast in this clade.

Description of *Candida phyllophila* Limtong and Kaewwichian sp. nov.

Growth in yeast extract malt extract (YM) broth: After 3 days at 25°C, cells are subglobose and ovoid to ellipsoidal (2.0–5.0 × 2.3–7.0 μm), and occur singly or in pairs (Fig. 2a). Budding is multilateral. Growth on

YM agar: After 3 days at 25°C, the streak culture is butyrous, white-colored, with a smooth surface and has an entire margin. Pseudohyphae and true hyphae are not formed in slide culture on corn meal agar after 7 days at 25°C. Ascospores were not formed by individual strains or strains paired on 5% malt extract agar, Fowell's acetate agar, Gorodkova agar or cornmeal agar after 4 weeks at 15 or 25°C.

Fermentation of D-glucose and D-galactose (slow) are positive but negative for maltose, sucrose, melibiose, lactose, raffinose and xylose. D-Glucose, sucrose, D-galactose, α-α-trehalose, maltose, melizitose, α-methyl-D-glucoside, cellobiose, salicin (weak), L-sorbose, D-xylose, D-ribose (weak), ethanol, glycerol, ribitol, xylitol, D-mannitol, D-glucitol, succinate, citrate, D-glucuronate, 2-keto-D-gluconate (weak), D-glucono-δ-lactone, N-acetyl glucosamine, cadaverine, ethylamine HCl and L-lysine HCl are assimilated, but inulin, raffinose, melibiose,

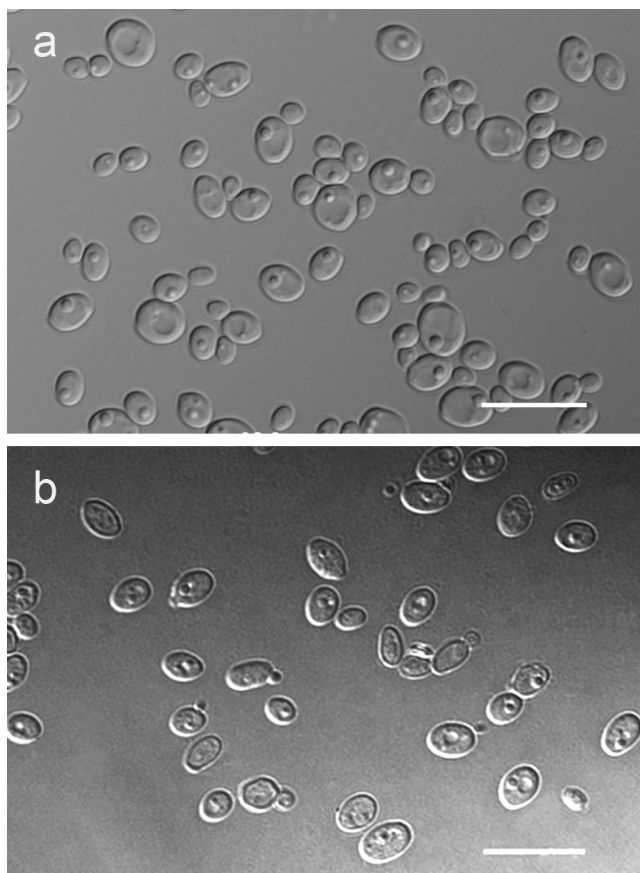


Fig. 2. Budding cells on YM agar after 3 days at 25°C of (a) *Candida phyllophila* sp. nov. (K59^T) and (b) *Candida vitiphila* sp. nov. (K70^T).

Scale bar, 10 μm.

lactose, soluble starch, L-rhamnose, L-arabinose, D-arabinose, methanol, erythritol, galactitol, *myo*-inositol, DL-lactate, 5-keto-D-gluconate, D-glucuronate, D-galacturonate, potassium nitrate and sodium nitrite are not. Growth on medium containing 50% (w/v) glucose, 60% (w/v) glucose or 10% (w/v) sodium chloride/5% (w/v) glucose is positive but negative for 16% (w/v) sodium chloride/5% (w/v) glucose. Growth with 0.01% cycloheximide and 0.1% cycloheximide is negative. Growth at 25, 30, 35 and 37°C is positive, but at 40°C is negative. Acid formation is present. Starch-like compounds are not produced. Diazonium blue B color and urease reactions are negative. The major ubiquinone is Q-8.

Holotype: K59^T is the holotype of *Candida phyllophila* (MB 801036). The strain was isolated from phylloplane of wine grape (*Vitis vinifera*, Family Vitaceae), collected in Kanchanaburi Province, Thailand. The living culture from type was deposited at the BIOTEC Culture Collection (BCC), National Center for Genetic

Engineering and Biotechnology (BIOTEC), Pathumthani, Thailand, as BCC 42662^T; NITE Biological Resources Center (NBRC), Department of Biotechnology, National Institute of Technology and Evaluation, Chiba, Japan, as NBRC 107776^T and Centraalbureau voor Schimmelcultures (CBS), Utrecht, the Netherlands, as CBS 12671^T.

Etymology: The species epithet *phyllophila* refers to the phylloplane where the strain was isolated.

Description of *Candida vitiphila* Limtong and Kaewwichian sp. nov.

Growth in yeast extract malt extract (YM) broth: After 3 days at 25°C, cells are subglobose and ovoid to ellipsoidal (1.6–4.0 × 2.8–6.4 μm), and occur singly or in pairs (Fig. 2b). Budding is multilateral. Growth on YM agar: After 3 days at 25°C, the streak culture is butyrous, white-colored, with a smooth surface and has an entire margin. Pseudohyphae and true hyphae are not formed in slide culture on corn meal agar after 7 days at 25°C. Ascospores were not produced on 5% malt extract agar, Fowell's acetate agar, Gorodkova agar or cornmeal agar after 4 weeks at 15 or 25°C.

Fermentation of D-glucose and D-galactose (slow) are positive but negative for maltose, sucrose, melibiose, lactose, raffinose and xylose. D-Glucose, sucrose, D-galactose, α-α-trehalose, maltose, melizitose, α-methyl-D-glucoside, cellobiose, salicin (weak), L-sorbose, D-xylose, D-ribose (weak), ethanol, glycerol, ribitol, xylitol, D-mannitol, D-glucitol, succinate, citrate, D-gluconate, 2-keto-D-gluconate (weak), D-glucono-δ-lactone, *N*-acetyl glucosamine, cadaverine, ethylamine HCl and L-lysine HCl are assimilated, but inulin, raffinose, melibiose, lactose, soluble starch, L-rhamnose, L-arabinose, D-arabinose, methanol, erythritol, galactitol, *myo*-inositol, DL-lactate, 5-keto-D-gluconate, D-glucuronate, D-galacturonate, potassium nitrate and sodium nitrite are not. Growth on medium containing 50% (w/v) glucose, 60% (w/v) glucose or 10% (w/v) sodium chloride/5% (w/v) glucose is positive but negative for 16% (w/v) sodium chloride/5% (w/v) glucose. Growth with 0.01% cycloheximide and 0.1% cycloheximide is negative. Growth at 25, 30, 35 and 37°C is positive, but at 40°C is negative. Acid formation is present. Starch-like compounds are not produced. Diazonium blue B color and urease reactions are negative. The major ubiquinone is Q-8.

Holotype: K70^T is the holotype of *Candida vitiphila* (MB 801037). The strain was isolated from the phylloplane of wine grapes (*Vitis vinifera*, Family Vitaceae),

collected in Kanchanaburi Province, Thailand. The living culture from type was deposited at the BIOTEC Culture Collection (BCC), National Center for Genetic Engineering and Biotechnology (BIOTEC), Pathumthani, Thailand, as BCC 42663^T; NITE Biological Resources Center (NBRC), Department of Biotechnology, National Institute of Technology and Evaluation, Chiba, Japan, as NBRC 107777^T and Centraalbureau voor Schimmelfcultures (CBS), Utrecht, the Netherlands, as CBS 12672^T.

Etymology: The species epithet *vitiphila* refers to *Vitis vinifera* where the strain was isolated.

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References

- Altschul, S. F., Madden, T. L., Schäffer, J. Z., Zhang, J., Zhang, Z., Miller, W., and Lipman, D. J. (1997) Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. *Nucleic Acids Res.*, **25**, 3389–3402.
- Andrews, J. H. and Harris, R. E. (2000) The ecology and biogeography of microorganisms on plant surfaces. *Annu. Rev. Phytopathol.*, **38**, 145–180.
- Chang, C. F., Liu, Y. R., Chen, S. F., Naumov, G. I., Naumova, E. S., and Lee, C. F. (2012) Five novel species of the anamorphic genus *Candida* in the *Cyberlindnera* clade isolated from natural substrates in Taiwan. *Antonie van Leeuwenhoek*, **102**, 9–21.
- de Azeredo, L. A. I., Gomes, E. A. T., Mendonca-Hagler, L. C., and Hagler, A. N. (1998) Yeast communities associated with sugarcane in Campos, Rio de Janeiro. *Brazil. Int. Microbiol.*, **1**, 205–208.
- Egli, C. M. and Henick-Kling, T. (2001) Identification of *Brettanomyces/Dekkera* species based on polymorphism in the rRNA Internal Transcribed Spacer Region. *Am. J. Enol. Vitic.*, **52**, 241–247.
- Felsenstein, J. (1985) Confidence limits on phylogenies: An approach using the bootstrap. *Evolution*, **39**, 783–791.
- Fonseca, A. and Inacio, J. (2006) Phylloplane yeasts. In *Biodiversity and Ecophysiology of Yeasts*, ed. by Rosa, C. and Peter, G., Springer-Verlag, Berlin/Heidelberg, pp. 263–301.
- Glushakova, A. M. and Chernov, I. Y. (2010) Seasonal dynamics of the structure of epiphytic yeast communities. *Microbiology*, **79**, 830–839.
- Glushakova, A. M., Yurkov, A. M., and Chernov, I. Y. (2007) Massive isolation of anamorphous ascomycete yeasts *Candida oleophila* from plant phyllosphere. *Microbiology*, **76**, 799–803.
- Inácio, J., Portugal, L., Spencer-Martins, I., and Fonseca, A. (2005) Phylloplane yeasts from Portugal: Seven novel anamorphic species in the Tremellales lineage of the Hymenomycetes (Basidiomycota) producing orange-coloured colonies. *FEMS Yeast Res.*, **5**, 1167–1183.
- Inácio, J., Rodrigues, M. G., Sobral, P., and Fonseca, A. (2004) Characterization and classification of phylloplane yeasts from Portugal related to the genus *Taphrina* and description of five novel *Lalaria* species. *FEMS Yeast Res.*, **4**, 541–555.
- Kaewwichian, R., Yongmanitchai, W., Kawasaki, H., and Limtong, S. (2012) *Metschnikowia saccharicola* sp. nov. and *Metschnikowia lopburiensis* sp. nov., two novel yeast species isolated from phylloplane in Thailand. *Antonie van Leeuwenhoek*, **102**, 743–751.
- Kimura, M. (1980) A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.*, **16**, 111–120.
- Koowadjanakul, N., Jindamorakot, S., Yongmanitchai, W., and Limtong, S. (2011) *Ogataea phyllophila* sp. nov., *Candida chumphonensis* sp. nov. and *Candida matranensis* sp. nov., three methylotrophic yeast species from phylloplane in Thailand. *Antonie van Leeuwenhoek*, **100**, 207–217.
- Kuraishi, H., Katayama-Fujimura, Y., Sugiyama, J., and Yokoyama, T. (1985) Ubiquinone systems in fungi. I. Distribution of ubiquinones in the major families of ascomycetes, basidiomycetes, and deuteromycetes, and their taxonomic implications. *Trans. Mycol. Soc. Jpn.*, **26**, 383–395.
- Lachance, M.-A. (2011a) *Clavispora* Rodrigues de Miranda (1979). In *The Yeasts, a Taxonomic Study*, 5th ed., Vol. 2, ed. by Kurtzman, C. P., Fell, J. W., and Boekhout, T., Elsevier, Amsterdam, pp. 349–353.
- Lachance, M.-A. (2011b) *Metschnikowia* Kamienski (1899). In *The Yeasts, a Taxonomic Study*, 5th ed., Vol. 2, ed. by Kurtzman, C. P., Fell, J. W., and Boekhout, T., Elsevier, Amsterdam, pp. 575–620.
- Lachance, M.-A., Boekhout, T., Scorzett, G., Fell, J. W., and Kurtzman, C. P. (2011) *Candida* Boekhout. In *The Yeasts, a Taxonomic Study*, 5th ed., Vol. 2, ed. by Kurtzman, C. P., Fell, J. W., and Boekhout, T., Elsevier, Amsterdam, pp. 987–1279.
- Landell, M. F., Billodre, R., Ramos, J. P., Leoncini, O., Vainstein, M. H., and Valente, P. (2010) *Candida aechmeae* sp. nov. and *Candida vrieseae* sp. nov., novel yeast species isolated from the phylloplane of bromeliads in Southern Brazil. *Int. J. Syst. Evol. Microbiol.*, **60**, 244–248.
- Limtong, S., Yongmanitchai, W., Tun, M. M., Kawasaki, H., and Seki, T. (2007) *Kazachstania siamensis* sp. nov., an ascomycetous yeast species from forest soil in Thailand. *Int. J. Syst. Evol. Microbiol.*, **57**, 419–422.
- Lu, H.-Z., Jia, J.-H., Wang, Q.-M., and Bai, F.-Y. (2004) *Candida asparagi* sp. nov., *Candida diospyri* sp. nov. and *Candida*

- qinlingensis* sp. nov., novel anamorphic, ascomycetous yeast species. *Int. J. Syst. Evol. Microbiol.*, **54**, 1409–1414.
- Nakase, T. and Suzuki, M. (1986) *Bullera megalospora*, a new species of yeast forming larger ballistospores isolated from dead leaves of *Oryza sativa*, *Miscanthus sinensis* and *Sasa* sp. in Japan. *J. Gen. Appl. Microbiol.*, **32**, 225–240.
- Nakase, T., Takashima, M., Itoh, M., Fungsin, B., Potacharoen, W., Atthasampunna, P., and Komagata, K. (2001) Ballistoconidium-forming yeasts found in the phyllosphere of Thailand. *Microbiol. Cult. Coll.*, **17**, 23–33.
- Phaff, H. J. and Starmer, W. T. (1987) Yeasts associated with plants, insects and soil. In *The Yeasts*, 2nd ed., ed. by Rose, A. H. and Harrison, J. S., Academic Press, London, pp. 123–180.
- Saitou, N. and Nei, M. (1987) The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.*, **4**, 406–425.
- Saksinchai, S., Suzuki, M., Lumyong, S., Ohkuma, M., and Chantawannakul, P. (2012) Two new species of the genus *Candida* in the *Zygoascus* clade, *Candida lundiana* sp. nov. and *Candida suthepensis* sp. nov., isolated from raw honey in Thailand. *Antonie van Leeuwenhoek*, **101**, 633–640.
- Slavikova, E., Vadkertiova, R., and Vranova, D. (2009) Yeasts colonizing the leaves of fruit trees. *Ann. Microbiol.*, **59**, 419–424.
- Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., and Kumar, S. (2011) MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.*, **28**, 2731–2739.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F., and Higgins, D. G. (1997) CLUSTAL_X windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.*, **24**, 4876–4882.
- White, T. J., Bruns, T., Lee, S., and Taylor, J. W. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols: A Guide to Methods and Applications*, ed. by Innis, M. A., Gelfand, D. H., Sninsky, J. J., and White, T. J., Academic Press, New York, pp. 315–322.
- Yamada, Y. and Kondo, K. (1973) Coenzyme Q system in the classification of the yeast genera *Rhodotorula* and *Cryptococcus*, and the yeast-like genera *Sporobolomyces* and *Rhodospiridium*. *J. Gen. Appl. Microbiol.*, **19**, 59–77.
- Yarrow, D. (1998) Methods for the isolation, maintenance and identification of yeasts. In *The Yeasts, a Taxonomic Study*, 4th ed., ed. by Kurtzman, C. P. and Fell, J. W., Elsevier, Amsterdam, pp. 77–100.