Candida xylanilytica sp. nov., a xylan-degrading yeast species isolated from Thailand

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Xylan is a major component of hemicellulose, which constitutes about 40% of plant biomass. Hydrolysis of xylan into simple sugars is one of the important steps in the conversion of lignocellulosic material to value-added products. During an investigation of cellulose- and xylan-degrading yeasts, two yeast strains that were able to use cellulose and xylan as sole carbon source were found to represent a phylogenetically distinct species in the Spathaspora clade. The closest species in terms of pairwise sequence similarity in the D1/D2 domain of the LSU rRNA gene was Candida subhashii. The novel species can be distinguished from the other species in the Spathaspora clade based on the ability to assimilate methanol and raffinose, growth in medium containing 60% glucose, and growth at 42°C. It ferments glucose but not other carbohydrates. The name Candida xylanilytica sp. nov. is proposed for this species. The type strain is KU-Xn11 T (=NBRC 106499T =BCC 34694T =CBS 11761T).

The Spathaspora clade was recognized by Nguyen et al. (2006) and comprised Spathaspora arborariae, Spathaspora passalidarum and five anamorphic species in the genus Candida. The xylose-fermenting species, Candida lyxosophilica and S. arborariae, were isolated from surface woodland soil in South Africa and rotting wood in Brazil, respectively, while Candida jeffriesii and S. passalidarum were found to associate with the gut of wood-boring beetles, Phrenapates bennetti in Panama and Odontotaenius disjunctus in Louisiana, USA (Cadete et al., 2009; Nguyen et al., 2006; van der Walt et al., 1987). The other three species of the Spathaspora clade do not ferment xylose. Candida insectamans and Candida materiae were found in frass of buprestid beetle larvae and rotting wood, respectively, while Candida subhashii was isolated from a fungal peritonitis patient in 2006 (Adam et al., 2009; Barbosa et al., 2009; van der Walt et al., 1972).

In the course of an investigation of cellulose- and xylan-degrading yeast in Thailand, two yeast strains which were able to use cellulose and xylan as sole carbon source were found to represent a novel species in the Spathaspora clade, based on analysis of the D1/D2 domains of the large-subunit (LSU) rRNA gene sequence. In the present study, a novel species in the genus Candida is described.

Strains KU-Xn11 and KU-Xn20 were isolated from decayed corn cobs and decayed grasses, respectively, at the National Corn and Sorghum Research Center, Nakhon Ratchasima, Thailand, by an enrichment technique using
sequences were compared pairwise by BLASTN search. Sequences of the D1/D2 domain of the LSU rRNA gene were aligned with the multiple alignment program CLUSTAL X version 1.83 (Thompson et al., 1997). Phylogenetic trees were reconstructed from the evolutionary distance data with Kimura’s two-parameter correction (Kimura, 1980) using the neighbour-joining method (Saitou & Nei, 1987) and by the maximum-parsimony method using the max-mini branch-and-bound algorithm of MEGA version 4 (Nei & Kumar, 2000; Tamura et al., 2007). Confidences for the phylogenetic tree were estimated from bootstrap analysis (1000 replicates) (Felsenstein, 1985).

The D1/D2 domains of the LSU rRNA gene of strains KU-Xn11 and KU-Xn20 have identical sequences which are present as two distinct copies in the genome. One copy has seven consecutive T nucleotides (copy 1, 569 nt), the other has eight T nucleotides (copy 2, 570 nt) in the same region. This may result from variability in tandem repeats of the rRNA gene. The closest species in terms of pairwise sequence similarity was Candida subhashii, with 5.6 % nucleotide substitution (32 bp) and 17 indels in copy 1, and 5.8 % nucleotide substitution (33 bp) and 16 indels in copy 2. The two strains showed greater than 1 % nucleotide substitution in the D1/D2 domain of the LSU rRNA gene and were presumed to represent a distinct species from Candida subhashii, according to the guidelines of Kurtzman & Robnett (1998). Phylogenetic analysis based on the D1/D2 domain of the LSU rRNA gene demonstrated that the two strains formed a cluster with Candida insectamans with high bootstrap support and were related to the other known species of the Spathaspora clade (Fig. 1).

The two strains proliferated by multilateral budding and pseudoaphyphae were abundant (Fig. 2). The strains were negative for Diazonium blue B and urease reactions and had Q-9 as the major ubiquinone. Lipid accumulated in cells grown in 5 % malt extract agar, cornmeal agar or YM agar after 5 days at 25 °C. Ascospores were not produced from individual strains or mixed culture on 5 % malt extract agar, cornmeal agar, Fowell’s acetate agar, diluted vegetable juice agar or YM agar after 5 weeks at 15 °C. Xylose fermentation was absent, similar to Candida insectamans, Candida materiae and Candida subhashii. The novel strains could be distinguished from the other species of the Spathaspora clade based on the ability to assimilate methanol and raffinose, growth in medium containing 60 % glucose and growth at 42 °C. Based on the data above, we concluded that the two strains represent a single novel species of the genus Candida in the Spathaspora clade, for which the name Candida xylanilytica sp. nov. is proposed.

Latin diagnosis of Candida xylanilytica Boonmak, Limtong, Jindamorakot, Am-In, Yongmanitchai, Suzuki, Nakase et Kawasaki sp. nov.

C. xylanilytica KU-Xn11T (AB523752)
Candida xylanilytica KU-Xn20 (AB523754)
Spathaspora arboriaef UFMG-HM19.1A (GQ149001)
Candida jeffriesi NRRL Y-27738 (AY520415)
Candida materiaef UFMG 07-C15.1B (FJ154790)
Spathaspora passalidarum NRRL Y-27907 (DQ109807)
Candida lyxosophilia NRRL Y-17539 (U76204)
Candida subhif AIH 10744 (EU386708)
Candida sp. GA1507 (FJ527144)
Candida insectamans NRRL Y-7786 (U45753)
Debaryomyces udeii NRRL Y-17354 (U45844)
Debaryomyces robertsiae NRRL Y-6670 (U45806)
Debaryomyces hansenii NRRL Y-7426 (U45808)
Candida psychrophila NRRL Y-17665 (U45813)
Debaryomyces nepalensis NRRL Y-7108 (U45839)
Saccharomyces cerevisiae NRRL Y-12632 (AY048154)

D-mannitol, D-glucitol, acidum DL-lacticum, acidum succinicum (lente vel nullum), acidum D-gluconicum (lente vel nullum), D-glucosaminum, N-acetyl-D-glucosaminum, 2-ketogluconicum, propanum-1,2-diolum (lente), xylanum et arbutinum assimilantur at non inulinum, melibiosum, galactosum, lactosum, l-sorbosum, l-rhamnosum, D-arabinosum, erythritol, galactitol, inositolum, acidum citricum, hexadecanum, xylitolum, 5-ketogluconicum, acidum D-glucoronicum, arabinitolum, D-glucono-1,5-lactonum, acidum D-galacturonicum nec butanum-2,3-diolum. Ethylaminum, l-lysinum et cadaverinum assimilantur at non nitricum nec nitrocum. Crescit sine vitaminis (exiguum). Crescit in 50% glucosum et 60% glucosum (exiguum). Non crescit in 0.01% cycloheximido, 0.1% cycloheximido nec 10% natrii chloridum/5% glucosum. Amylum non formatur. Diazonium caeruleum B non respondens. Ureum non hydrolysatur. Maxima temperatura crescentiae: 42 °C. Proportio molaris guanini + cytosini in acido deoxyribonucleico: 38.3, 39.2 mol% (per HPLC). Ubiquinonum majus: Q-9. Typus strips KU-Xn11T (=NBRC 106499T =BCC 34694T =CBS 11761T) isolatus ex putrefacio Zea mays axis, Nakhon Ratchasima Provincia, Thailandia, conservatur in

Fig. 1. Phylogenetic tree based on sequences of the D1/D2 domain of the LSU rRNA gene, showing the positions of the two strains of Candida xylanilytica sp. nov., with respect to closely related species. The neighbour-joining (NJ) phylogenetic tree was reconstructed from evolutionary distance data corrected by the two-parameter transformation of Kimura (1980). The maximum-parsimony (MP) phylogenetic tree was reconstructed using the max-min branch-and-bound algorithm (Nei & Kumar, 2000). Numbers indicate percentages of bootstrap sampling, derived from 1000 samples. Bars, 0.02 substitutions per nucleotide position (NJ) or 5 steps (MP).

Fig. 2. Morphology of vegetative cells of strain KU-Xn11T. (a) Vegetative cell grown in YM broth for 3 days at 25 °C. (b) Pseudomycelia produced on slide culture with cornmeal agar after 7 days at 25 °C. (c) Pseudomycelia produced on Dalmau plate culture with cornmeal agar after 14 days at 25 °C. Bars, 10 μm.
hydrolysis is positive. Diazonium blue B and urease is positive but weakly positive with 60% glucose. Starch glucose is negative. Growth on medium with 50% glucose salicin, D-xylose, L-arabinose, D-ribose, methanol (slow), D-arabinose, erythritol, galactitol, inositol, citric acid, ethylamine, L-lysine and cadaverine are assimilated, but butane-2,3-diol, nitrate and nitrite are not assimilated. Growth in vitamin-free medium is weak. Growth at 42°C but not at 45°C. Growth on medium containing 0.01% cycloheximide and medium containing 10% NaCl and 5% glucose is negative. Growth on medium with 50% glucose is positive but weakly positive with 60% glucose. Starch formation and gelatin liquefaction are negative. TWEEN 80 hydrolysis is positive. Diazonium blue B and urease reactions are negative. DNA G+C content is 38.3–39.2 mol%. Major ubiquinone is Q-9.

The type strain, KU-Xn11\textsuperscript{T} (=NBRC 106499\textsuperscript{T} =BCC 34694\textsuperscript{T} =CBS 11761\textsuperscript{T}), was isolated from decayed corn cobs at the National Corn and Sorghum Research Center, Nakhon Ratchasima, Thailand. A living culture of the type is deposited at the NITE Biological Resources Center (NBRC), Department of Biotechnology, National Institute of Technology and Evaluation (NITE), Chiba, Japan, as NBRC 106499\textsuperscript{T}, the BIOTEC Culture Collection (BCC), National Center for Genetic Engineering and Biotechnology (BIOTEC), Pathumthani, Thailand, as BCC 34694\textsuperscript{T}, and the Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands, as CBS 11761\textsuperscript{T}.

**Description of Candida xylanilytica** Boonmak, Limtong, Jindamorakot, Am-In, Yongmanitchai, Suzuki, Nakase & Kawasaki sp. nov.

*Candida xylanilytica* (xy.lan.i.ly’ti.ca. N.L. n. *xylanum* xylan, a plant polysaccharide; Gr. adj. *lutikos* -ē -on able to loose, able to dissolve; N.L. fem. adj. *xylanilytica* hydrolysing xylan).

After growth in YM broth for 3 days at 25°C, loose floating islets and heavy sediment are present. Cells proliferate by multilateral budding and are spheroidal, ovoidal, ellipsoidal or elongate, 3.2–9.5 × 3.2–23.2 μm and occur singly, in pairs or in small clusters. Pseudomyelidia are produced. After growth in YM agar for 1 month at 25°C, streak culture is smooth, butyrous, cream–coloured and umbonate with ciliate margin. Sometimes pulvinate wrinkled colonies are obtained from smooth colonies. In Dalmau plate culture on cornmeal agar after 5 days at 25°C, pseudomyelidia are produced abundantly. Blastocoididia are formed in clusters. Ascospores are not produced from individual strains or mixed culture on 5% malt extract agar, cornmeal agar, Fowell’s acetate agar, diluted vegetable juice agar or YM agar after 5 weeks at 15°C. Fermentation of glucose is positive but fermentation of galactose, sucrose, maltose, lactose, raffinose, trehalose, cellobiose and xylose is negative. Glucose, sucrose, raffinose (weakly positive or negative), trehalose, maltose, melezitose, N-methyl D-glucoside, soluble starch, cellobiose, salicin, D-xylose, L-arabinose, D-ribose, methanol (slow), ethanol, glycerol, ribitol, D-mannitol, D-glucitol, DL-lactic acid, succinic acid (latent or negative), D-gluconic acid (latent or negative), D-glucosamine, N-acetyl-D-glucosamine, 2-ketogluconic acid, propane-1,2-diol (latent), xylan, ethylamine, L-lysine and cadaverine are assimilated, but inulin, melibiose, galactose, lactose, L-sorbose, L-rhamnose, D-arabinose, erythritol, galactitol, inositol, citric acid, hexadecane, xylitol, 5-ketogluconic acid, D-glucuronic acid, arabinol, D-glucono-1,5-lactone, D-galacturonic acid, butane-2,3-diol, nitrate and nitrite are not assimilated. Growth in vitamin-free medium is weak. Growth at 42°C but not at 45°C. Growth on medium containing 0.01% cycloheximide and medium containing 10% NaCl and 5% glucose is negative. Growth on medium with 50% glucose is positive but weakly positive with 60% glucose. Starch formation and gelatin liquefaction are negative. TWEEN 80 hydrolysis is positive. Diazonium blue B and urease reactions are negative. DNA G+C content is 38.3–39.2 mol%. Major ubiquinone is Q-9.

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**References**


