Candida thermophila sp. nov., a novel thermophilic yeast isolated from soil

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Yeast strain Y94T, which is capable of growth at high temperature, was isolated from soil in Korea. Characteristics of the strain include asexual reproduction by multilateral budding, the absence of extracellular starch-like compounds, a negative Diazonium blue B colour reaction, and the absence of arthropores, ballistoconidia and ascospores; the strain can therefore be placed in the genus Candida. A maximum growth temperature of 50–51 °C, along with certain other physiological characteristics, and a unique 26S rDNA partial sequence separate this strain from other ascomycetous yeasts. Taken together, these results suggest that the strain is a novel species and the name Candida thermophila sp. nov. (type strain is Y94T = JCM 10994T = KCCM 50661T) is proposed.

Keywords: thermophilic yeast, taxonomy, Candida thermophila sp. nov.

Thermotolerant and/or thermophilic micro-organisms are very useful for certain industrial processes (Banat et al., 1998; Banat & Marchant, 1995; Kadam & Schmidt, 1997). The production of biological materials at high temperatures rather than the customary practice makes it possible to reduce the risk of contamination and the operation costs of maintaining growth temperatures in large-scale systems, and to increase the rate of productivity, etc. (Nolan et al., 1994). For these reasons, many efforts have been made to seek or develop thermotolerant and/or thermophilic strains (Gera et al., 1997; Kiran Sree et al., 2000).

In the present study, a novel thermophilic yeast, strain Y94T, is described on the basis of physiological and chemosystematic studies, as well as phylogenetic analysis of the D1/D2 domain of the large-subunit rRNA coding gene (LSU rDNA). Results of the present study showed that strain Y94T could be distinguished from other species in the genus Candida Berkhout (Meyer et al., 1998), as well as other ascomycetous yeasts. The name Candida thermophila sp. nov. is proposed for this novel yeast.

Strain Y94T was isolated on YM agar plates (1%, w/v, glucose; 0.5% peptone; 0.3% yeast extract; 0.3% malt extract; 2% agar) in which the pH was adjusted to 3.8 with 5 M HCl at 50 °C. Morphological, physiological and biochemical characteristics were examined according to the methods of Yarrow (1998). The maximum growth temperature was determined in YM broth using metal block baths. Coenzyme Q was extracted, purified and identified by the method of Nakase & Suzuki (1988). The DNA G + C composition was determined by the method of Tamaoka & Komagata (1984).

Genomic DNA isolation and PCR amplification of the D1/D2 region of the 26S rDNA were performed according to the protocols of Kurtzman & Robnett (1998). The amplified fragments were purified using the QIAquick PCR purification kit (Qiagen) and directly sequenced with an ABI Taq DyeDeoxy Terminator Cycle Sequencing kit and an ABI 310 DNA sequencer (Applied Biosystems). The resulting sequences were aligned automatically with the multiple-sequence alignment program CLUSTAL W (Thompson et al., 1994) and were manually corrected. Phylogenetic relationships were inferred with the PHYLIP program package (Felsenstein, 1993). A distance matrix was obtained using the DNADIST program and a phylogenetic tree was constructed by the neighbour-joining method (Saitou & Nei, 1987) with the NEIGHBOR program. Bootstrap values (Felsenstein, 1985) were calculated from 1000 replicates. Saccharomyces cerevisiae was included as the designated outgroup in the analysis. Other related sequences were obtained from the GenBank database (Kurtzman & Robnett, 1997, 1998).
The genus *Candida* is composed of species with a broad range of phenotypic properties (Meyer et al., 1998). Analyses of LSU rDNA D1/D2 domain sequences from most of the *Candida* species showed that the genus *Candida* is not monophyletic and that species are widely distributed among the ascosporic yeasts (Kurtzman & Robnett, 1998).

The results of D1/D2 region sequence analysis revealed that strain Y94T was phylogenetically closely related to *Pichia angusta*, *Pichia phildendrea*, *Pichia minuta* var. *minuta* and *Pichia minuta* var. *nonfermentans* (Fig. 1). However, the sequence of the 26S rDNA variable domain differed from those of the *Pichia* species by more than 1% divergence (7 nt substitutions) and sexual reproduction was not detected. Kurtzman & Robnett (1998) suggested that conspecific yeast strains normally have less than 1% nucleotide substitution in the D1/D2 LSU rDNA. On the basis of that criterion, strain Y94T appears to be a distinct species compared to other ascomycetous yeasts.

Strain Y94T was further distinguished from known *Pichia* species by various physiological characteristics (Kurtzman, 1998). Strain Y94T could be differentiated from *P. angusta* because it was unable to assimilate sucrose, maltose, methyl α-D-glucoside or melizitose. The ability of strain Y94T to assimilate cellobiose, salicin and arbutin separated it from *P. phildendrea*. It differed from *P. minuta* var. *minuta* and *P. minuta* var. *nonfermentans* by its ability to assimilate L-sorbose, meso-erythritol and D-gluconic acid. Strain Y94T was unable to assimilate succinic acid, which was an additional property that could discriminate it from the type strains of *P. angusta*, *P. phildendrea*, *P. minuta* var. *minuta* and *P. minuta* var. *nonfermentans*.

The most striking difference between the four above-mentioned species and strain Y94T was the maximum growth temperature. Strain Y94T was able to grow at 50–51 °C, whereas *P. angusta*, *P. phildendrea*, *P. minuta* var. *minuta* and *P. minuta* var. *nonfermentans* were not. Furthermore, strain Y94T grew most actively at 30–35 °C, rather than at the usual optimum growth temperature of 25–30 °C, which is common to many other yeasts (data not shown).

**Latin diagnosis of Candida thermophila sp. nov.**

*In liquido YM, post dies 3 ad 25 °C, cellulae vegetative globosae vel spheroidales, 23–38 × 2.5–4.6 μm, singulae, per gennationem multilaterealem reproduntur. Post unum mensem ad 25 °C, pellicula non formatur, sedimentum formatur. Cultura in agar YM, post dies 3 ad 25 °C, butyroza, glabra, candida aut creamea. In agar farina Zea mays confection post 7 dies ad 25 °C, pseudomycelium nullum. Amylum non format. Diazonium caeruleum B non respondens. Ureum non fingitur. Sexualis coniunctio non manifesta. Materialia amylolidea non formanunt. Vitaminae externae ad crescentiam necessaria sunt. Crescit in medio cum 50% glucosio (exiguo) neque medio cum 60% glucosio. Crescit in medio 10% sodii chlorid et 5% glucosi. Crescere potest cum 0.01% et 0.1% cycloheximid. Non crescit in medio 1% acido acetico addito. Maxima temperatura crescentiae: 50–51 °C. Systema coenzymatis Q-7 adest. Proportio molaris G + C in acid deoxyribonucleico: 45.9 mol% (per HPLC). D-Glucosum et α,α-trehalosum (lente et exiguo) fermentantur at non D-galactosum, sucrosum, maltosum, cellobiosum, melibiosum, lactosum, raffinosum, inulimum nec ammonium, D-Glucosum, L-sorbosum, D-ribosum, D-xylosum, D-arabinosum (exiguo), L-rhamnosum (exiguo), α,α-trehalosum, cellobiosum, salicinum, glycercinum, erythritolum, ribitolum, xylitolum, D-glucitolum, D-mannitolum, galactitolum (exiguo), D-glucono-δ-lactonum, D-glucurate, acidum citricum, methanolum (exiguo) et alcohol aethylicum assimilantur at non D-galactosum, D-glucosaminum, L-arabinosum, sucrosum, maltosum, methyl α-glucosidum, melibiosum, lactosum, raffinosum, melitosum, inulimum, ammonium, inositolum, acidum D-glucuronicum, D-altaractum nec acidum succinicum. Assimilatio kalii nitritatis (exiguo), sodii nitrosi (exiguo), D-glucosaminum (exiguo), ethylaminum, L-lysini et cadaverini, as non creatinii nec creatinini. Typus stirps Y94T ex terra (china clay), Goryung, Korea isolatae. In collectionibus culturarum quas Japanae Collection of Micro-

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**Fig. 1.** Phylogenetic tree resulting from analysis of the D1/D2 regions of the 26S rDNA sequences. The numbers represent the confidence level (%) from 1000 replicate bootstrap sampling. Bar, 0.1 nucleotide substitutions per nucleotide position.
The following are not assimilated: -arabinose, sucrose, maltose, methyl -xylose, -arabinose (weak), -rhamnose (weak), -trehalose, cellobiose, salicin, -glucitol, -lactone, -glucose, -lactate and sucrose, starch, inositol, -glucuronate, DL-lactate and succinate. Nitrate, nitrite and -glucosamine are weakly utilized. Ethylamine, l-lysine and cadaverine are utilized strongly. Creatine, creatinine and d-tryptophan are not utilized. The genomic DNA G+C content of strain Y94T is 45-9 mol%. The major isoprenoid quinone is ubiquinone Q-7. The type strain is Y94T (= JCM 10994T = KCCM 50661T). It was isolated from a soil sample (china clay) obtained from Goryung, Korea.

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References


