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NOTE

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

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Yeast strain Y94^T, 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 arthrospores, 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 Y94^T = JCM 10994^T = KCCM 50661^T) 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 Y94^T, 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 Y94^T 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 Y94^T 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).

Abbreviation: LSU rDNA, large-subunit rRNA coding gene.

The GenBank accession number for the 26S rDNA D1/D2 domain sequence of Y94^T is AF283568.

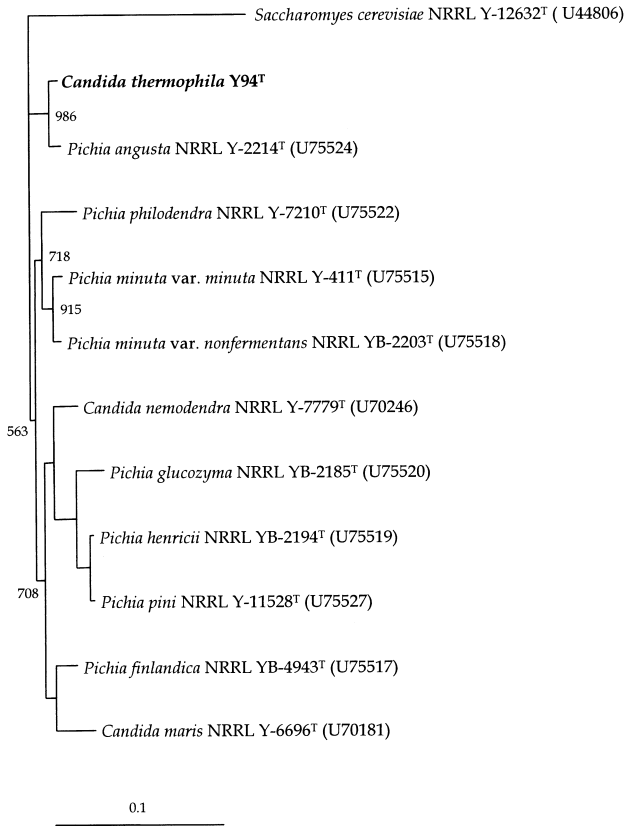

Fig. 1. Phylogenetic tree resulting from analysis of the D1/D2 regions of the 26S rDNA sequences. The numbers represent the confidence level (%) from 1000 replicate bootstrap sampling. Bar, 0·1 nucleotide substitutions per nucleotide position.

The 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 Y94^T was phylogenetically closely related to *Pichia angusta*, *Pichia philodendra*, *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 Y94^T appears to be a distinct species compared to other ascomycetous yeasts.

Strain Y94^T was further distinguished from known *Pichia* species by various physiological characteristics (Kurtzman, 1998). Strain Y94^T could be differentiated from *P. angusta* because it was unable to assimilate sucrose, maltose, methyl α-D-glucoside or melezitose. The ability of strain Y94^T to assimilate cellobiose, salicin and arbutin separated it from *P. philodendra*. 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 Y94^T was unable to assimilate succinic acid, which was an additional property that could discriminate it from the type strains of *P. angusta*, *P. philodendra*, *P. minuta* var. *minuta* and *P. minuta* var. *nonfermentans*.

The most striking difference between the four above-mentioned species and strain Y94^T was the maximum growth temperature. Strain Y94^T was able to grow at 50–51 °C, whereas *P. angusta*, *P. philodendra*, *P. minuta* var. *minuta* and *P. minuta* var. *nonfermentans* were not. Furthermore, strain Y94^T 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*, 2·3–3·8 × 2·5–4·6 μm, *singulae*, *per gemmationem multilateraleum reproducentes*. *Post unum mensem ad* 25 °C, *pellicula non formatur*, *sedimentum formatur*. *Cultura in agaro YM*, *post dies* 3 *ad* 25 °C, *butyrosa*, *glabra*, *candida aut cremea*. *In agaro farina Zea mays confecto post* 7 *dies ad* 25 °C, *pseudomycelium nullum*. *Amylum non formatur*. *Diazonium caeruleum* B *non respondens*. *Ureum non fingitur*. *Sexualis coniunctio non manifesta*. *Materia amyloidea non formantur*. *Vitaminae externae ad crescentiam necessaria sunt*. *Crescit on medio cum* 50% *glucoso* (*exigue*) *neque medio cum* 60% *glucoso*. *Crescit in medio* 10% *sodii cloridii 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 acido deoxyribonucleico*: 45·9 mol% (*per* HPLC). D-*Glucosum et* α,α-*trehalosum* (*lente et exigue*) *fermentantur at non* D-*galactosum*, *sucrosum*, *maltosum*, *cellobiosum*, *melibiosum*, *lactosum*, *raffinosum*, *inulinum nec amylum*. D-*Glucosum*, L-*sorbosum*, D-*ribosum*, D-*xylosum*, D-*arabinosum* (*exigue*), L-*rhamnosum* (*exigue*), α,α-*trehalosum*, *cellobiosum*, *salicinum*, *glycerinum*, *erythritolum*, *ribitolum*, *xylitolum*, D-*glucitolium*, D-*mannitolum*, *galactitolum* (*exigue*), D-*glucono-δ-lactonum*, D-*gluconate*, *acidum citricum*, *methanolum* (*exigue*) *et alcohol aethylicum assimilantur at non* D-*galactosum*, D-*glucosaminum*, L-*arabinose*, *sucrosum*, *maltosum*, *methylum* α-*glucosidum*, *melibiosum*, *lactosum*, *raffinosum*, *melezitosum*, *inulinum*, *amylum*, *inositolum*, *acidum* D-*glucuronicum*, DL-*acidum lacticum nec acidum succinicum*. *Assimilatio kallii nitratis* (*exigue*), *sodii nitrosi* (*exigue*), D-*glucosaminum* (*exigue*), *ethylaminum*, L-*lysini et cadaverini*, *as non creatini nec creatinini*. *Typus stirps* Y94^T *ex terra* (china clay), Goryung, Korea *isolataest*. *In collectionibus culturarum quas* Japan Collection of Micro-

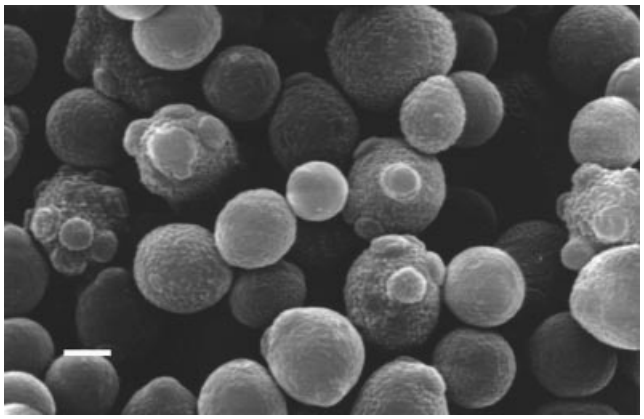

Fig. 2. Scanning electron micrograph of *Candida thermophila* sp. nov. grown in YM agar for 3 d at 25 °C. Cell division is by multilateral budding. Bar, 1 μm.

organisms, Wako, *Japonia* (= JCM 10994^T) *et* Korean Culture Center of Microorganisms, Seoul, Korea (= KCCM 50661^T).

Description of *Candida thermophila* sp. nov.

Candida thermophila (ther.mo′phi.la. Gr. adj. *thermos* warm; Gr. adj. *philos* loving; N.L. adj. *thermophila* heat-loving).

After 3 d growth in YM broth at 25 °C, the cells are globose to spheroidal, 2·3–3·8 × 2·5–4·6 μm and occur singly. Multilateral budding is observed (Fig. 2). A sediment is formed after 4 weeks. No pellicle is formed. Slant culture on YM agar after 3 d at 25 °C is butyrous, glistening and white to cream-coloured. In slide culture on corn meal agar after 3 weeks at 25 °C, pseudomycelium is not formed. Production of amyloid compounds and the Diazonium blue B reaction are negative. Urea is not hydrolysed. Sexual reproduction is not observed on 5 % malt extract agar, acetate agar, yeast extract-malt extract agar, corn meal agar, potato-dextrose agar or V8 agar. No growth occurs in vitamin-free medium or in the presence of 1 % acetic acid. Cells grow in the presence of 0·01 % (w/v) and 0·1 % (w/v) cycloheximide. Weak growth occurs on 50 % glucose agar, but not on 60 % glucose agar. Growth in 10 % (w/v) NaCl plus 5 % (w/v) glucose broth is positive. The maximum temperature for growth is 50–51 °C in YM broth. Splitting of arbutin is detected. Glucose and trehalose (slow and weak) are fermented, but not D-galactose, sucrose, maltose, cellobiose, melibiose, lactose, raffinose, inulin or starch. The following carbon compounds are assimilated: D-glucose, L-sorbose, D-ribose, D-xylose, D-arabinose (weak), L-rhamnose (weak), α,α-trehalose, cellobiose, salicin, glycerol, erythritol, ribitol, xylitol, D-glucitol, D-mannitol, galactitol (weak), glucono-δ-lactone, D-gluconate, citrate, methanol (weak) and ethanol. The following are not assimilated: D-galactose, D-glucosamine, L-arabinose, sucrose, maltose, methyl α-glucoside, melibiose, lactose, raffinose, melezitose, inulin, starch, inositol, D-glucuronate, DL-lactate and succinate. Nitrate, nitrite and D-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 Y94^T is 45·9 mol %. The major isoprenoid quinone is ubiquinone Q-7. The type strain is Y94^T (= JCM 10994^T = KCCM 50661^T). It was isolated from a soil sample (china clay) obtained from Goryung, Korea.

Acknowledgements

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