Candida gosینگica sp. nov., an anamorphic ascomycetous yeast closely related to *Scheffersomyces spartinae*

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During surveys on yeast diversity in forest soils from Taiwan and Thailand, ten yeast strains isolated from different samples were found to have similar molecular and physiological characteristics. Sequence analysis of small subunit (SSU) rDNA, the D1/D2 domain of large subunit (LSU) rDNA and internal transcribed spacer (ITS)-5.8S rDNA demonstrated that these strains were closely related to *Scheffersomyces spartinae*. The novel strains could be differentiated from *S. spartinae* by a 0.9% sequence divergence (5 substitutions, 0 gaps) in the D1/D2 domain of LSU rDNA, a 1.5% divergence (8 substitutions, 0 gaps) in the ITS-5.8S rDNA and a 0.7% divergence (12 substitutions, 2 gaps) in the SSU rDNA. The novel strains also showed specific patterns of electrophoretic karyotypes that differed from that of *S. spartinae*. Therefore, a novel yeast species, *Candida gosینگica* sp. nov., is proposed to accommodate these strains. The type strain SJ7S11^T (=BCRC 23194^T=CBS 11433^T) was assigned and deposited in the Bioresource Collection and Research Center (BCRC), Food Industry Development and Research Institute, Hsinchu, Taiwan, and Centraalbureau voor Schimmelcultures (CBS), Utrecht, The Netherlands.

Pichia spartinae, proposed by Ahearn *et al.* (1970), is characterized by the production of coenzyme Q-9 and the presence of hat-shaped ascospores. The species has since been transferred to a new genus, *Yamadazyma*, established by Billon-Grand (1989) and based on the production of coenzyme Q-9. Kurtzman & Robnett (1998) pointed out that the three Q-9-producing species assigned to the genus *Yamadazyma* by Billon-Grand, *Pichia acaciae*, *Pichia guilliermondii* and *P. spartinae*, were clustered among several clades in the phylogenetic tree, and found that they were quite closely related to members of the genus *Debaryomyces*. Because of the uncertain circumscription of the genera *Yamadazyma* and *Pichia* and their unresolved relationships with other genera that produce coenzyme

Q-9, Kurtzman & Suzuki (2010) resolved the species of these genera into 12 well-supported clades based on the sequences of the D1/D2 domain of the large subunit (LSU) and small subunit (SSU) rDNAs. According to this analysis, 12 genera were proposed, one for each clade. *Pichia spartinae* was transferred to the genus *Scheffersomyces* (Kurtzman & Suzuki, 2010). In this report, a novel anamorphic yeast closely related to *Scheffersomyces spartinae* is described.

During an investigation of the yeast community associated with soil in Taiwan, seven strains were isolated from forest soil samples in January, February, April, August, and October of 2006. The novel strains were found to possess similar morphological, physiological and molecular characteristics and were closely related to *S. spartinae*. Additional strains isolated from Thailand were confirmed to be conspecific to those from Taiwan. These strains, which form the basis for the proposal of a new species in this study, are listed in Table 1. Strain isolation was performed by the method described by Lee *et al.* (2008). Yeasts were grown on YM agar at 24 °C for 3 days, followed by preservation at –70 °C and/or on YM agar at 4 °C. The additional strains from Thailand (Table 1) were isolated according to the methods described by Limtong *et al.*

Abbreviations: ITS, internal transcribed spacer; LSU, large subunit; SSU, small subunit.

The GenBank/EMBL/DDBJ accession numbers for the LSU rDNA D1/D2, SSU rDNA and ITS region for *Candida gosینگica* sp. nov. SJ7S11^T are EF460550, FJ153193 and FJ152110, respectively. The GenBank accession numbers for all strains of *C. gosینگica* sp. nov. examined in this study are presented in Supplementary Table S1 available in IJSEM Online.

A supplementary table is available with the online version of this paper.

(2009). Isolated strains were morphologically, physiologically and biochemically characterized using standard methods for current yeast taxonomy (Yarrow, 1998). The strains used for the examination of ascospores were incubated on McClary's acetate agar, cornmeal agar, malt extract agar and V8 agar (Yarrow, 1998), individually or as pairwise mixtures on the sporulation medium. The cultures were observed for ascospore production every week for a month at 18 °C.

Yeast DNA was extracted using a Biokit Genome DNA Extraction kit according to the manufacturer's protocol (Biokit Co.). The D1/D2 LSU rDNA, SSU rDNA and ITS regions were amplified by PCR and sequenced using the primer pairs NL1 and NL4 (Kurtzman & Robnett, 1998), P1 and NS8 (Suzuki & Nakase, 2002) and ITS1 and ITS4 (White *et al.*, 1990), respectively. The resulting sequences were compared with those of reference organisms that were retrieved from the GenBank database. The sequences for phylogenetic analysis were aligned automatically by the CLUSTAL_X 1.83 multiple sequence alignment program (Thompson *et al.*, 1997). Phylogenetic and molecular evolutionary analyses were conducted using the MEGA version 4.0 software package (Kumar *et al.*, 2004), and evolutionary distances were calculated using the neighbour-joining method with the Kimura two-parameter distance measure. Confidence values were estimated from bootstrap analyses of 1000 replicates (Felsenstein, 1985). *Wickerhamia fluorescens* NRRL YB-4819^T was the designated outgroup in the analyses; other related sequences were obtained from GenBank. The full list of the GenBank/EMBL/DDBJ accession numbers for the LSU rDNA, SSU rDNA and ITS-5.8S sequences reported in this paper is given in Supplementary Table S1 (see IJSEM Online).

To examine the electrophoretic karyotypes of the yeasts by PFGE, agarose plugs containing intact chromosomal DNA were prepared according to the method described by Carle & Olson (1985) and modified by Lee *et al.* (2009a, b). Agarose blocks were cut to fit the wells of a 1 % agarose gel

(Amresco) and the plug pieces were inserted and sealed in place with 1 % agarose. Gels were equilibrated in 0.5 × TBE (45 mM Tris/borate, 1 mM EDTA, pH 8.0) for 1 h before electrophoresis.

Chromosomal DNA bands were separated on 1 % agarose gel in 0.5 × TBE buffer in a rotating-field electrophoresis machine (Rotaphor 6.0; Biometra). The electrophoresis program was completed in two steps, with a total running time of 94 h: Step 1, field switch time of 120–360 s for 24 h at 120 V; Step 2, 360–1200 s for 70 h at 70 V. The temperature of the running buffer was maintained at 13 °C throughout electrophoresis. After electrophoresis, the gels were stained with 0.5 µg ethidium bromide ml⁻¹ for 30 min, destained with tap water for 30 min and then photographed under UV illumination. Intact chromosomal DNA of *Saccharomyces cerevisiae* YPH 80 purchased from Biometra was used as a DNA marker.

Sequence comparison

All the strains belonging to the proposed novel species were found to share identical ITS and SSU rDNA sequences. Strain SF5S11 differed by only two substitutions (2 gaps) in the D1/D2 LSU rDNA sequence from the other strains, indicating that these strains are conspecific. Phylogenetic analysis based on the sequences of D1/D2 LSU rDNA showed that *S. spartinae* was the most closely related species (Fig. 1) to the novel strains, which differed from *S. spartinae* by a 0.9 % sequence divergence (5 substitutions, 0 gaps), which is at the high end of the divergence range usually observed for members of the same species (Kurtzman & Robnett, 1998). The ITS-5.8S and SSU rDNA sequences of the novel strains showed a 1.5 % divergence (8 substitutions, 0 gaps) and a 0.7 % divergence (12 substitutions, 2 gaps), respectively, from those of *S. spartinae* NRRL Y-7322^T. These results clearly indicated that the new strains were representatives of a novel species closely related to *S. spartinae*.

Electrophoretic karyotypes

In order to further elucidate the taxonomic relationships of the novel species with *S. spartinae*, the electrophoretic karyotype of each novel strain was determined and compared with that of *S. spartinae* NRRL Y-7322^T. The strains representing the novel species were found to be quite different to *S. spartinae* NRRL Y-7322^T in terms of electrophoretic karyotypes, with 6–8 chromosomal bands ranging from 745–3000 kb (Fig. 2). Strains SC10S07, SG4S01 and SA8S03 exhibited polymorphisms in chromosomal DNA size and band number (Fig. 2, lanes 10, 11 and 12) when compared with the other novel strains. It is possible that strains in the same species with different PFGE patterns could be differentiated between strains at the species level (Querol *et al.*, 1992; Stoltenburg *et al.*, 1992). The electrophoretic karyotypes of these strains were very similar or identical to each other but were distinctly different from those of their closest relative *S. spartinae*.

Table 1. Yeast strains examined in this study

Strains	Source
<i>P. spartinae</i> NRRL Y-7322 ^T	<i>Spartina alterniflora</i> , Louisiana, USA
<i>Candida goslingica</i> sp. nov.	
SJ7S11 ^T	Soil, Nanto, Taiwan
SF5S11	Soil, Hualien, Taiwan
SC2S03	Soil, Taoyuan, Taiwan
SC10S07	Soil, Taoyuan, Taiwan
SG2S06	Soil, Nanto, Taiwan
SG4S01	Soil, Nanto, Taiwan
SA8S03	Soil, Miaoli, Taiwan
CP1	Soil, Thep Sathit, Chaiyaphum, Thailand
LY20	Soil, Phu Ruea, Loei, Thailand
LY24	Soil, Phu Ruea, Loei, Thailand

Fig. 1. Neighbour-joining phylogenetic tree based on the D1/D2 divergent domain of the LSU rDNA sequences of *Candida goslingica* sp. nov. SJ7S11^T and related species. Bootstrap values (%) >50 from 1000 samples are shown. GenBank accession numbers are given in parentheses. Bar, 0.01 nt substitutions per site. *Wickerhamia fluorescens* NRRL YB-4819^T (U45719) was used as the outgroup.

NRRL Y-7322^T (Fig. 2). The results described above indicate that these strains represent a separate species closely related to *S. spartinae*.

Taxonomy

None of the novel strains examined in this study produced ascospores or exhibited conjugation in common sporulation medium either alone or in pairwise mixtures. In the absence of a sexual cycle, the molecular characteristics described above were used for species delineation of the proposed novel species. As evidenced by analysis of DNA sequences and electrophoretic karyotypes, these strains represent a new species closely related to *S. spartinae*. In addition, the novel strains all exhibit similar assimilation patterns. The strains of the novel species could be differentiated from *S. spartinae* by their inability to grow on citrate, *N*-acetyl-D-glucosamine or 10 % NaCl/5 % glucose at 37 °C and by their ability to grow on glycerol, D-gluconate and vitamin-free medium. Based on this evidence, a novel species, *Candida goslingica* sp. nov., is

proposed to accommodate these strains with the type strain SJ7S11^T.

Latin diagnosis of *Candida goslingica* Lee sp. nov.

In medio agar YM post dies 3 ad 25 °C, cellulae subglobosae vel ellipsoideae (3.1–5.5 et 3.8–6.7 µm), singulae aut binae, per gemmationem reproducentes. *Cultura in agar* YM post dies 3 ad 25 °C, parva, glabra, nitida, alba et margine glabra. Post unum mensem ad 25 °C, annulus, sedimentum formatur. Pseudomycelium formantur. Glucosum, maltosum, methyl α-D-glucosidum, sucrosum, cellobiosum et melezitium fermentantur, at non galactosem, trehalosum, melibiosum, lactatum, raffinosem, inulinum, amyllum solubile nec xylosum. Assimilantur D-glucosum, L-sorbosem, sucrosum, maltosum, trehalosum, methyl α-D-glucosidum, cellobiosum, salicinum, arbutinum, melezitium, ribitolium, xylitolium, D-glucitolium, D-mannitolium, D-glucono-1,5-lactonum (exiguum), 2-ketogluconatum, DL-lactatum, succinatum, ethanolum, propane-1,2-diolum et butano-2,3-diolum. Non assimilantur D-galactosum, D-glucosaminum, D-ribosum, D-xylosum, L-arabiosum, D-arabiosum, L-rhamnosum, melibiosum, lactosum, raffinosem, inulinum, amyllum solubile, glycerolum, erythritolum, L-arabitolium, galactitolium, myo-inositolium, 5-ketogluconatum, D-gluconatum, D-glucuronatum, acidum galacturonicum, acidum citratum, methanolum et *N*-acetyl-D-glucosaminum. Assimilantur ethylaminum, L-lysinum et cadaverinum. Non assimilantur kalii nitratum, natrium nitrosum et creatinum. 25 °C, 30 °C et 35 °C crescit, neque 37 °C. Non crescit in medio 0.01 % cycloheximido addito. Non crescit in medio 1 % acido acético addito. Non crescit in medio cum 50 % glucoso. Non crescit in substrato 10 % sal/5 % glucosum continente. Vitamina externa ad crescentiam necessaria non sunt. Amyllum non formatur. Acidum aceticum non formatur. Ureum non hydrolysatur. Diazonium caeruleum B est negativum.

Typus SJ7S11^T (=BCRC 23194^T=CBS 11433^T) *isolatus ex terea*, in Nanto, Taiwan. *Conservatur* in Bioresource Collection and Research Center (BCRC), Food Industry Development and Research Institute, Hsinchu, Taiwan; et Centraalbureau voor Schimmelcultures (CBS), Utrecht, The Netherlands.

Fig. 2. Electrophoresis karyotypes of strains of *Candida goslingica* sp. nov. and related type strains. Lanes: 1 and 13, marker (*Saccharomyces cerevisiae* YPH80); 2, *Scheffersomyces spartinae* NRRL Y-7322^T; 3, *Candida goslingica* sp. nov. SJ7S11^T; 4, SF5S11; 5, SC2S03; 6, SG2S06; 7, CP1; 8, LY20; 9, LY24; 10, SC10S07; 11, SG4S01; 12, SA8S03.

Description of *Candida gosینگica* Lee sp. nov.

Candida gosینگica (go.sin.gi'ca. N.L. fem. adj. *gosینگica* of or belonging to Gosing, Nanto, Taiwan, where the yeast was originally isolated).

After growth on YM agar for 3 days at 25 °C, cells are subglobose to ellipsoidal, $3.1\text{--}5.5 \times 3.8\text{--}6.7\text{ }\mu\text{m}$ long, and occur singly or in pairs (Fig. 3a). Asexual reproduction is by multilateral budding. Streak culture is smooth, glistening and white in colour, with an entire margin. After 1 month of incubation in YM broth at 25 °C, sediment is observed. Pseudomycelia are formed in Dalmau plate cultures on cornmeal agar after 2 weeks incubation (Fig. 3b). Glucose, maltose, methyl α -D-glucoside, sucrose, cellobiose and melzitose are fermented, but galactose, trehalose, melibiose, lactose, raffinose, inulin, starch and xylose are not fermented. D-Glucose, L-sorbose, sucrose, maltose, trehalose, methyl α -D-glucoside, cellobiose, salicin, arbutin, melezitose, ribitol, xylitol, D-glucitol, D-mannitol, D-glucono-1,5-lactone (weak), 2-keto-D-gluconate, DL-lactate, succinate, ethanol, propane-1,2-diol and butane-2,3-diol are assimilated, but D-galactose, D-glucosamine, D-ribose, D-xylose, L-arabinose, D-arabinose, L-rhamnose, melibiose, lactose, raffinose, inulin, soluble starch, glycerol, erythritol, L-arabinitol, galactitol, *myo*-inositol, 5-keto-D-gluconate, D-gluconate, D-glucuronate, D-galacturonic acid,

citrate, methanol and *N*-acetyl-D-glucosamine are not assimilated. Ethylamine, L-lysine and cadaverine are utilized, while potassium nitrate, sodium nitrite and creatine are not utilized. Growth occurs on YM agar at 25 °C, 30 °C and 35 °C, but not at 37 °C. Growth in the presence of 0.01 % cycloheximide is negative, as is growth on 1 % acetic acid, 50 % glucose and 10 % sodium chloride plus 5 % glucose agar. Growth in vitamin-free medium is positive. Production of starch-like compounds is absent. Acetic acid production on chalk agar is negative. Diazonium blue B reaction and urease activity are negative.

The type strain, SJ7S11^T (=BCRC 23194^T=CBS 114833^T), was isolated from soil in Nanto, Taiwan and deposited in the Bioresource Collection and Research Center (BCRC), Food Industry Development and Research Institute, Hsinchu, Taiwan, and Centraalbureau voor Schimmelcultures (CBS), Utrecht, The Netherlands.

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References

- Ahearn, D. G., Yarrow, D. & Meyers, S. P. (1970). *Pichia spartinae* sp. n. from Louisiana marshland habitats. *Antonie van Leeuwenhoek* **36**, 503–508.
- Billon-Grand, G. (1989). A new ascosporeogenous yeast genus: *Yamadazyma* gen. nov. *Mycotaxon* **35**, 201–204.
- Carle, G. F. & Olson, M. V. (1985). An electrophoretic karyotype for yeast. *Proc Natl Acad Sci U S A* **82**, 3756–3760.
- Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.
- Kumar, S., Tamura, K. & Nei, M. (2004). MEGA3: Integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief Bioinform* **5**, 150–163.
- Kurtzman, C. P. & Robnett, C. J. (1998). Identification and phylogeny of ascomycetous yeasts from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences. *Antonie van Leeuwenhoek* **73**, 331–371.
- Kurtzman, C. P. & Suzuki, M. (2010). Phylogenetic analysis of ascomycete yeasts that form coenzyme Q-9 and the proposal of the new genera *Babjeviella*, *Meyerozyma*, *Priceomyces*, and *Scheffersomyces*. *Mycoscience* **51**, 2–14.
- Lee, C. F., Liu, C. H., Young, S. S. & Chang, K. S. (2008). *Kazachstania jiaimicus* sp. nov., an ascomycetous yeast species isolated from soil in Taiwan. *FEM Yeast Res* **8**, 114–118.
- Lee, C. F., Yao, C. H., Liu, Y. R., Hsieh, C. W. & Young, S. S. (2009a). *Lachancea dasiensis* sp. nov., an ascosporeogenous yeast isolated from soil and leaves in Taiwan. *Int J Syst Evol Microbiol* **59**, 1818–1822.
- Lee, C. F., Yao, C. H., Liu, Y. R., Young, S. S. & Chang, K. S. (2009b). *Kazachstania wufongensis* sp. nov., an ascosporeogenous yeast isolated from soil in Taiwan. *Antonie van Leeuwenhoek* **95**, 335–341.
- Limtong, S., Yongmanitchai, W., Kawasaki, H. & Fujiyama, K. (2009). *Wickerhamomyces edaphicus* sp. nov. and *Pichia jaroonii* sp. nov., two ascomycetous yeast species isolated from forest soil in Thailand. *FEM Yeast Res* **9**, 504–510.

Fig. 3. Morphology of *Candida gosینگica* sp. nov. SJ7S11^T as determined by light microscopy. (a) Budding cells grown on YM agar for 3 days at 25 °C. Bar, 10 μm . (b) Pseudohyphae grown on cornmeal agar for 2 weeks at 25 °C. Bar, 20 μm .

Querol, A., Barrio, E. & Ramon, D. (1992). A comparative study of different methods of yeast strain characterization. *Syst Appl Microbiol* **15**, 439–446.

Stoltenburg, R., Klinner, U., Ritzefeld, P., Zimmermann, M. & Emeis, C. C. (1992). Genetic diversity of the yeast *Candida utilis*. *Curr Genet* **22**, 441–446.

Suzuki, M. & Nakase, T. (2002). A phylogenetic study of ubiquinone-7 species of the genus *Candida* based on 18S ribosomal DNA sequence divergence. *J Gen Appl Microbiol* **48**, 55–65.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible

strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.

White, T. J., Bruns, T., Lee, S. & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols: a Guide for Methods and Applications*, pp. 315–322. Edited by M. A. Innis, D. H. Gelfand, J. J. Sninsky & T. J. White. New York: Academic Press.

Yarrow, D. (1998). Methods for the isolation, maintenance and identification of yeasts. In *The Yeasts, a Taxonomic Study*, 4th edn, pp. 77–100. Edited by C. P. Kurtzman & J. W. Fell. Amsterdam: Elsevier.