Cryptococcus keelungensis sp. nov., an anamorphic basidiomycetous yeast isolated from the sea-surface microlayer of the north-east coast of Taiwan

Chin-Feng Chang,1 Chung-Fu Lee2 and Shiu-Mei Liu1

1Institute of Marine Biology, National Taiwan Ocean University, 2 Pei-Ning Road, Keelung 20224, Taiwan, ROC
2Department of Applied Science, National Hsinchu University of Education, 521 Nanda Road, Hsinchu 30014, Taiwan, ROC

Strain SN-82T was isolated from the sea-surface microlayer at Keelung on the north-east coast of Taiwan. Sequence analysis of the D1/D2 domain of the 26S rDNA of strain SN-82T suggested that this strain is related to the aerius clade in the Filobasidiales. Phenotypic characteristics such as the absence of sexual structures and ballistoconidia, the assimilation of myo-inositol and d-glucuronate, the inability to ferment glucose, the possession of coenzyme Q-10 and positive Diazonium blue B and urease reactions also indicated that this strain belongs to the genus Cryptococcus. However, divergences of more than 3.6% were observed in the D1/D2 domain when compared with other described Cryptococcus species, which indicated that the isolated yeast represents a previously unrecognized member of this genus. Therefore, the novel yeast species Cryptococcus keelungensis sp. nov. is proposed, with strain SN-82T (=BCRC 23107T =JCM 14893T) as the type strain.

Members of the genus Cryptococcus are commonly isolated from seawater (Yamasato et al., 1974; Fell, 1976; Hagler & Ahearn, 1987; Nagahama et al., 2003). This basidiomycetous yeast genus is characterized mainly by the lack of sexual spores and ballistoconidia, utilization of D-glucuronate, the absence of fermentative ability, the presence of xylose in cell hydrolysates, positive Diazonium blue B and urease tests and the possession of coenzyme Q-9 or Q-10 (Fell & Statzell-Tallman, 1998). The genus Cryptococcus is also known to be polyphyletic (Fell et al., 2000; Scorzetti et al., 2002) and its members are heterogeneous in terms of their nutritional abilities.

The sea-surface microlayer (SSML) is a thin film of liquid at the interface of the sea and the atmosphere and is of considerable importance in exchanges that occur between them. Conditions at the SSML are harsh, with high levels of visible and UV radiation and a high concentration of pollutants (Varnam & Evans, 2000). Therefore, the SSML has often been considered an extreme environment for micro-organisms and may contain unusual species and taxa (Maki, 2002).

During a survey of the yeast community associated with the SSML in Taiwan, strain SN-82T was isolated together with more than 200 other yeast strains at Keelung on the north-east coast (25°09’27”N 120°09’22”E). Based on the sequences of the internal transcribed spacer (ITS) region and the 26S rDNA D1/D2 domain, these 200 isolates were identified as belonging to 36 yeast species of the genera Aureobasidium (1), Candida (10), Cryptococcus (5), Hanseniaspora (1), Lodderomyces (1), Pichia (3), Pseudozyma (1), Rhodosporidium (4), Rhodotorula (5), Sporobolomyces (1), Sporidiobolus (1), Trichosporon (1) and Yarrowia (2). Four strains isolated from the SSML might represent undescribed taxa. For the novel anamorphic basidiomycetous isolate SN-82T, the name Cryptococcus keelungensis sp. nov. is proposed on the basis of its morphological, physiological and molecular characteristics.

Samples were collected in the morning during four field campaigns (November 2005, February 2006, May 2006 and August 2006). Seawater from the SSML was collected with a metal screen (Garrett, 1967) and a glass plate (Harvey & Burzell, 1972). One litre of each seawater sample was concentrated to 1 ml by centrifugation at 2000 g for 20 min. After serial dilution of the concentrated seawater samples, subsamples (100 μl each) were spread onto acidic yeast extract-malt extract (YM) agar plates (pH 4.5–5.0) (Difco) supplemented with 0.01% (w/v) chloramphenicol and then incubated at 25 °C in the dark. All of the colonies

Abbreviations: ITS, internal transcribed spacer; SSML, sea-surface microlayer.

The GenBank/EMBL/DDBJ accession numbers for the 26S rDNA D1/D2 and ITS region sequences of strain SN-82T are EF621562 and EF621565, respectively.
that appeared on the plates over a period of 3–5 days were transferred to YM broth for growth. The strains were then purified by repeated streaking of an isolated colony onto YM agar followed by incubation at 25 °C. Thereafter, the strains were maintained at 4 °C. For long-term preservation, cell suspensions were stored at –80 °C in broth cultures that were supplemented with 30 % (w/v) glycerol. Isolated strains were characterized morphologically, physiologically and biochemically using standard methods for current yeast taxonomy (Yarrow, 1998). Ubiquinones were extracted and purified following the method of Yamada & Kondo (1973) and then determined by HPLC as described previously (Nakase & Suzuki, 1988). The DNA base composition was determined by HPLC after enzymic digestion of the DNA to deoxyribonucleosides as described by Tamaoka & Komagata (1984).

The 26S rDNA D1/D2 domain and ITS regions were amplified and sequenced by PCR using the primer pairs F63 (5′-GCATATCAAAGCGGAAGAAG-3′) and LR3 (5′-GGTCCGTGTTTCAAGACG-3′) and ITS1 (5′-TCCGTAGGGAAACTCGG-3′) and ITS4 (5′-TCCCGGGTTATGGATATGC-3′), respectively (Scorzetti et al., 2002). The resulting sequences were compared with those of reference organisms that were retrieved from GenBank. Sequences for phylogenetic analysis were aligned automatically with the program CLUSTAL_X 1.83 (Thompson et al., 1997). Phylogenetic and molecular evolutionary analyses were conducted with MEGA version 3.1 (Kumar et al., 2004) and evolutionary distances were calculated using the neighbour-joining method with Kimura’s two-parameter distance measure. Confidence values were estimated from bootstrap analyses of 1000 replicates. Cryptococcus aquaticus CBS 5443T and Mrakia gelida CBS 5272T were the designated outgroup in the analyses.

Phylogenetic analysis based on sequences of the 26S rDNA D1/D2 domain showed that the novel strain belongs to the arius clade in the Filobasidiales (Fig. 1) as described by Fonseca et al. (2000) and Scorzetti et al. (2002). Within the arius clade, the novel strain occupied a relatively isolated position with Cryptococcus sp. SDY 170 (3.6 % divergence; 6 substitutions, 16 gaps), Cryptococcus sp. SDY 235 and SDY 169 (3.8 % divergence; 7 substitutions, 16 gaps), Filobasidium sp. CBS 10190 (3.7 % divergence; 6 substitutions, 17 gaps) and Filobasidium sp. CBS 10189 (4 % divergence; 7 substitutions, 17 gaps) and formed a subclade of its nearest relatives. Strain SN-82T and an unnamed taxon, represented by strains isolated in Portugal from acid mine drainage (Cryptococcus sp. strains SDY 169, 170, 235; Gadanho et al., 2006) and plant leaves (Filobasidium sp. strains CBS 10188, 10189, 10190), formed an independent cluster with other arius strains in the Filobasidiales; strain SN-82T has sufficiently different sequences to be considered a member of a separate species from these yeast strains. These different isolation sources also illustrate that members of the genus Cryptococcus are heterogeneous in terms of their nutritional abilities and have been isolated from diverse habitats. Physiologically, strain SN-82T could be distinguished from these related taxa as indicated in Table 1. Based on these differences, it is concluded that strain SN-82T represents a novel species, Cryptococcus keelungensis sp. nov.

Latin diagnosis of Cryptococcus keelungensis
Chang et Liu sp. nov.

In medio agaro YM post dies 3 ad 25 °C, cellularae spheroidae vel ovoideae (3.3–5.9 × 2.9–4.4 μm), singulae aut binae. Cultura in agaro YM post dies 3 ad 25 °C, parva, glabra, nitida, cremea

Fig. 1. Neighbour-joining tree based on the D1/D2 divergent domain of large-subunit rDNA sequences of Cryptococcus keelungensis sp. nov. SN-B2T and related species. Bootstrap values (%) above 60 % from 1000 samples are shown. Accession numbers are given in parentheses. Bar, 0.01 substitutions per site. Cryptococcus aquaticus CBS 5443T and Mrakia gelida CBS 5272T were used as outgroups.
Table 1. Selected characteristics of strain SN-82T and type strains of related Cryptococcus species

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assimilation of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-Sorbose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>w</td>
<td>d/+</td>
</tr>
<tr>
<td>D-Glucosamine</td>
<td>+</td>
<td>+</td>
<td>w</td>
<td>+</td>
<td>-</td>
<td>w</td>
</tr>
<tr>
<td>D-Arabinose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>w</td>
<td>d/+</td>
</tr>
<tr>
<td>L-Rhamnose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>w</td>
<td>d/+</td>
</tr>
<tr>
<td>Sucrose</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>w</td>
</tr>
<tr>
<td>Methyl α-D-glucoside</td>
<td>+</td>
<td>-</td>
<td>/d</td>
<td>+</td>
<td>-</td>
<td>w</td>
</tr>
<tr>
<td>Melibiose</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>w</td>
</tr>
<tr>
<td>Lactose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Raffinose</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Melezitose</td>
<td>+</td>
<td>d</td>
<td>d</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Starch</td>
<td>w</td>
<td>d/+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>w</td>
</tr>
<tr>
<td>Glycerol</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Erythritol</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>w</td>
<td>-</td>
</tr>
<tr>
<td>Ribitol</td>
<td>+</td>
<td>w/d</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>w</td>
</tr>
<tr>
<td>Xylitol</td>
<td>+</td>
<td>w/d</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>w</td>
</tr>
<tr>
<td>L-Arabitol</td>
<td>+</td>
<td>+</td>
<td>d</td>
<td>-</td>
<td>-</td>
<td>w</td>
</tr>
<tr>
<td>D-Glucitol</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>D-Mannitol</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Galactitol</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>D-Glucono-1,5-lactone</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>D-Galacturonic acid</td>
<td>+</td>
<td>+</td>
<td>d</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Succinate</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>d</td>
<td>-</td>
<td>w/d</td>
</tr>
<tr>
<td>Citrate</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Ethanol</td>
<td>w</td>
<td>d/w</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>w</td>
</tr>
<tr>
<td>Nitrile</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>L-Lysine</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Growth in the absence of vitamins</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Growth at/in:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>35 °C</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Acetic acid (1%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>D-Glucose (50 and 60%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Starch formation</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>w</td>
</tr>
</tbody>
</table>

Cryptococcus keelungensis sp. nov.

Cryptococcus keelungensis (kee.lung.en’is. N.L. nom. masc. adj. keelungensis referring to Keelung in Taiwan, where the organism was first isolated).

After growth on YM agar for 3 days at 25 °C, cells are spherical to ovoid, 3.3–5.9 μm long by 2.9–4.4 μm wide, occurring singly or in pairs (Fig. 2). Streak culture is smooth, glistening and cream coloured, with an entire margin. After 1 month incubation in YM broth at 25 °C, pellets and sediment are observed. No mycelia or pseudomycelia are formed in Dalmau plate cultures on cornmeal agar after 2 weeks incubation. Fermentation is absent. D-Glucose, D-galactose (weak), L-sorbose, D-glucosamine, D-ribose, D-xylitol, L- and D-arabinose, L-rhamnose, sucrose, maltose, trehalose, methyl α-D-glucoside, cellobiose, salicin, arbutin, lactose, raffinose, melezitose, soluble starch (weak), glycerol, ribitol, xylitol, L-arabitol, D-glucitol, D-mannitol, galactitol, myo-inositol, D-glucono-1,5-lactone, 2-keto-D-gluconate, 5-keto-D-gluconate, D-glucuronate, D-galacturonic acid, succinate, ethanol (weak) and N-acetyl-D-glucosamine are assimilated. Melibiose, inulin, propane-1,2-diol and butano-2,3-diol. Assimilantur kalii nitratum. Non assimilantur natrium nitrosus, L-lysinum et creatinum. Vitaminae externae ad crescentiam necessariae sunt. Non crescit in medio 0.1 % cycloheximido addito. Non crescit in medio 1 % acido acetic addito. Non crescerer potest in 10 % NaCl. Non crescit in medio cum 50 % glucose. Materia amyloidea idio phila formantur. Acidum aceticum haud formatum. Reactiones Diazonium caeruletum B et ureasii positivae. 25 °C et 35 °C crescit, necitque 40 °C. Ubiquinonum primus Q-10. G+C acidic deoxyribonucleati 54.8 mol% (per HPLC). Typus SN-82T (=CBS 10876T =BCRC 23107T = JCM 14893T ) isolatus ex aquamarinus, in Keelung, Taiwan.

Description of Cryptococcus keelungensis Chang & Liu sp. nov.

Cryptococcus keelungensis (kee.lung.en’is. N.L. nom. masc. adj. keelungensis referring to Keelung in Taiwan, where the organism was first isolated).

After growth on YM agar for 3 days at 25 °C, cells are spherical to ovoid, 3.3–5.9 μm long by 2.9–4.4 μm wide, occurring singly or in pairs (Fig. 2). Streak culture is smooth, glistening and cream coloured, with an entire margin. After 1 month incubation in YM broth at 25 °C, pellets and sediment are observed. No mycelia or pseudomycelia are formed in Dalmau plate cultures on cornmeal agar after 2 weeks incubation. Fermentation is absent. D-Glucose, D-galactose (weak), L-sorbose, D-glucosamine, D-ribose, D-xylitol, L- and D-arabinose, L-rhamnose, sucrose, maltose, trehalose, methyl α-D-glucoside, cellobiose, salicin, arbutin, lactose, raffinose, melezitose, soluble starch (weak), glycerol, ribitol, xylitol, L-arabitol, D-glucitol, D-mannitol, galactitol, myo-inositol, D-glucono-1,5-lactone, 2-keto-D-gluconate, 5-keto-D-gluconate, D-glucuronate, D-galacturonic acid, succinate, ethanol (weak) and N-acetyl-D-glucosamine are assimilated. Melibiose, inulin, D-Galacturonic acid, succinate, ethanol (weak) and N-acetyl-D-glucosamine are assimilated. Melibiose, inulin, propane-1,2-diol et butano-2,3-diol. Assimilantur kalii nitratum. Non assimilantur natrium nitrosus, L-lysinum et creatinum. Vitaminae externae ad crescentiam necessariae sunt. Non crescit in medio 0.1 % cycloheximido addito. Non crescit in medio 1 % acido acetic addito. Non crescerer potest in 10 % NaCl. Non crescit in medio cum 50 % glucose. Materia amyloidea idio phila formantur.

Fig. 2. Morphology of vegetative cells of Cryptococcus keelungensis sp. nov. SN-82T grown on YM agar for 3 days at 25 °C. Bar, 10 μm.
erythritol, DL-lactate, citrate, methanol, propane-1,2-diol and butane-2,3-diol are not assimilated. Potassium nitrate is utilized. Sodium nitrate, L-lysine and creatine are not utilized. Does not grow in vitamin-free medium. Growth in the presence of 0.1% cycloheximide, 1% acetic acid or 10% sodium chloride is inhibited and negative. Does not grow on 50% glucose-yeast extract agar. Produces starch-like compounds. Acetic acid production is negative. Positive for the Diazonium blue B reaction and urease activity. Grows at 25 and 35 °C, but not at 40 °C on YM agar. The major ubiquinone is Q-10.

The type strain is SN-82T (=CBS 10876T = BCRC 23107T = JCM 14893T), isolated from seawater collected from the SSML in Keelung, Taiwan. The nuclear DNA G + C content of the type strain is 54.8 mol% (by HPLC).

References


