Cryptococcus taeanensis sp. nov., a new anamorphic basidiomycetous yeast isolated from a salt farm

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Cryptococcus taeanensis, a new anamorphic yeast species originating from a salt farm on the Taean peninsula in Korea, is described. Strain 3-12T grew by budding, contained ubiquinone Q-10 and xylose in cell hydrolysates, utilized D-glucuronate and did not ferment D-glucose. A molecular phylogenetic analysis based on the large-subunit rRNA D1/D2 domain and ITS region sequences placed C. taeanensis near Auriculibuller fuscus and Bullera japonica, recently proposed taxa of the Tremellales. However, these species were distinguishable based on standard physiological tests used for yeast identification, with characteristics including the assimilation of L-sorbose, absence of ballistoconidia, no arbutin hydrolysis and no growth in the presence of 0.01 % cycloheximide. The isolate exhibited the typical physiology of the genus Cryptococcus Vuillemin, but its large-subunit rRNA D1/D2 domain sequence was clearly distinct from previously described species in the genus. Therefore, on the basis of these results, Cryptococcus taeanensis sp. nov. is proposed; the type strain is 3-12T (=KCTC 17149T=CBS 9742T).

The genus Cryptococcus Vuillemin is characterized by the lack of sexual spores and ballistoconidia, utilization of D-glucuronate, absence of fermentative ability, presence of xylose in cell hydrolysates, positive Diazonium blue B and urease reactions and the possession of coenzyme Q-9 or Q-10 (Fell & Statzell-Tallman, 1998). Members of the genus are found in a wide variety of habitats, and the genus has been recognized as markedly heterogeneous on the basis of a number of biochemical and molecular studies (Fell et al., 1995; Fell & Statzell-Tallman, 1998; Vancanneyt et al., 1994). More recently, several novel Cryptococcus strains isolated from plants and soil appear to be phylogenetically close to the Cryptococcus laurentii complex (Golubev et al., 2003; Sato et al., 1999; Scorzetti et al., 2002; Vishniac, 2002).

During a survey of the yeast community associated with salt farms in Korea, many basidiomycetous yeast strains were isolated from soil collected from the Taean peninsula and characterized by partial 26S rRNA gene sequencing. Comparative partial 26S rRNA gene sequence analysis showed that one strain, designated 3-12T, exhibited a specific phylogenetic association with taxa related to the Tremellales, while also displaying over 2% sequence divergence from established species. A further comparison of the phenotypic properties of the new isolate and its levels of sequence similarity with Cryptococcus species revealed that the isolate should be assigned to a novel species, for which the name Cryptococcus taeanensis sp. nov. is proposed.

Strain 3-12T was isolated from a soil sample collected from a salt farm on the Taean peninsula in Korea. One gram of each soil sample was suspended in 30 ml sterile water and stirred for 10 min. Then, 100 μl of the suspension was spread onto YM agar (Difco) supplemented with 5 μg gentamicin ml⁻¹ and 16 μg chloramphenicol ml⁻¹. Next, the strain was purified by streaking an isolated colony onto the YM agar without antibiotics and incubating at 25 °C. Thereafter, the strain was maintained at 4 °C; the cell suspensions were stored at −80 °C in broth cultures supplemented with 10% (w/v) glycerol, for long-term maintenance. The methods used to determine the morphological, physiological and biochemical properties were as described by Yarrow (1998). All assimilation tests were performed twice, in separate experiments. The results were read after 1 and 3 weeks incubation. Coenzyme Q was prepared and analysed as described by Nakase & Suzuki (1988). The presence of xylose in the cell wall was investigated by a TLC analysis, as described by Komagata & Suzuki (1987). Isolation of

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The GenBank/EMBL/DDBJ accession numbers for the 26S rRNA D1/D2 domain and ITS region sequences of Cryptococcus taeanensis 3-12T are AY422719 and AY686645, respectively. A phylogenetic tree based on analysis of ITS region (including 5.8S rRNA gene) sequences showing the position of C. taeanensis 3-12T in relation to taxa of the Tremellales is available in IJSEM Online.
The resultant sequences were compared with those of reference organisms retrieved from the GenBank database. The sequences were aligned automatically by using the multiple-sequence alignment program CLUSTAL W (Thompson et al., 1994), then corrected manually. Phylogenetic analyses were performed with the PHYLIP 3.5c package (Felsenstein, 1993), and the evolutionary distances were calculated by using the program DNA DIST according to the algorithm of Jukes & Cantor (1969). In the analysis, bootstrap correction (Jukes & Cantor, 1969) was used for all the isolates. The majority of the isolates grown over a temperature range of 10–30 °C and in the presence of 10% NaCl + 5% glucose; it was also, a mesophile and moderately halotolerant yeast, in agreement with the temperature and salinity of its salt-farm habitat. A solar saltern consists of shallow ponds connected in a sequence of increasingly saline brines, and it is used for the commercial production of salt from sea water. As such, with regard to this ecosystem, the isolate seemingly originated from the coastal marine environment and would appear to have adapted to or survived in a salt-farm environment.

The phylogenetic analysis based on the D1/D2 domain sequence showed that C. taeanensis belongs to the Bulleromyces clade in the Tremellales (Fig. 1) that includes species able to produce ballistospores together with species that do not present this characteristic (Scorzetti et al., 2002). C. taeanensis was also found to form a distinct lineage with Auriculibuller fuscus and Bullera japonica, with a 77% bootstrap confidence level, in contrast to other Cryptococcus species. The closest relatives of C. taeanensis were A. fuscus and B. japonica, which are ballistoconidia-forming species, as described in a recent study by Sampaio et al. (2004). However, when testing the ability of C. taeanensis to produce ballistoconidia with various media (malt agar, potato dextrose agar, corn meal agar and morphology agar), no ballistoconidia were observed. Moreover, C. taeanensis did not produce a brownish pigment when grown on potato.

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\begin{align*}
\text{Cryptococcus } & \text{ sp. CBS 8372 (AF444410)} \\
\text{Cryptococcus flavescens } & \text{ CBS 942T (AB035042)} \\
\text{Cryptococcus } & \text{ sp. CBS 8358 (AF444387)} \\
\text{Cryptococcus aurous } & \text{ CBS 318T (AB035041)} \\
\text{Bullera japonica } & \text{ CBS 2013T (AF444760)} \\
\text{Auriculibuller fuscus } & \text{ PYCC 5690T (AF444762)} \\
\text{Cryptococcus taeanensis } & \text{ KCTC 17149T (AY422719)} \\
\text{Cryptococcus nemorosus } & \text{ VKM Y-2906T (AF472625)} \\
\text{Cryptococcus perniciosus } & \text{ VKM Y-2905T (AF472624)} \\
\text{Papiliotrema bandomii } & \text{ IGC 5472T (AF416642)} \\
\text{Cryptococcus cellulolyticus } & \text{ CBS 8294T (AF075525)} \\
\text{Bullera pseudoalba } & \text{ CBS 7227T (AF075504)} \\
\text{Bullera unica } & \text{ CBS 8290T (AF075524)} \\
\text{Bulleromyces albus } & \text{ CBS 501T (AF075500)} \\
\text{Fellomyces polyborus } & \text{ CBS 6072T (AF189859)} \\
\text{Tremella encephala } & \text{ CBS 6968 (AF189867)}
\end{align*}
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![Fig. 1. Dendrogram showing phylogenetic relationship between Cryptococcus taeanensis sp. nov. 3-12T (=KCTC 17149T=CBS 9742T) and type strains of related species of the Bulleromyces clade (Tremellales), based on 26S rRNA D1/D2 domain sequences. Bootstrap values were calculated from 1000 trees and values less than 50% were omitted. GenBank/EMBL/DBJ accession numbers are given in parentheses. Bar, nucleotide substitutions per site. Outgroup species were Tremella encephala CBS 6968 and Fellomyces polyborus CBS 6072T.](image-url)
dextrose agar, in contrast to A. fuscus and B. japonica. In a comparison of the 26S rRNA D1/D2 domain sequences, C. taeanensis exhibited 12 base substitutions among 607 nucleotide positions with A. fuscus and 14 base substitutions with B. japonica.

To study the phylogenetic position of C. taeanensis more precisely, the ITS region (including 5-8S rRNA gene) sequence was compared with published sequences of strains related to C. taeanensis. The resulting phylogenetic relationships based on the ITS sequences confirmed a close phylogenetic relationship among C. taeanensis, A. fuscus and B. japonica (see Supplementary Fig. A available in IJSEM Online). In addition, the close relationship among Papilotrema bandonii, A. fuscus and B. japonica shown in Supplementary Fig. A agrees well with that described by Sampaio et al. (2004). C. taeanensis differed from A. fuscus by 45 base substitutions and nine indels in the ITS region, while it differed from B. japonica by 49 base substitutions and 10 base indels.

Scorzetti et al. (2002) recommended a combined sequence analysis of the D1/D2 domain and ITS region for the species identification of basidiomycetous yeasts. As such, the sequence similarities of both the D1/D2 domain and the ITS region between the isolate and related taxa were less than 99%, thereby indicating distinct species.

Based on the conventional criteria examined, as well as the molecular phylogenetic analysis, the novel isolate was assigned to the anamorphic basidiomycetous yeast genus Cryptococcus Vuillemin (Fell & Statzell-Tallman, 1998). However, the novel isolate did not split arbutin and was unable to grow in the presence of 0-01% cycloheximide, in contrast to A. fuscus and B. japonica, its nearest phylogenetic neighbours. In addition, C. taeanensis was distinct from A. fuscus based on its ability to assimilate L-sorbos as a sole carbon source (Sampaio et al., 2004). Therefore, the presented results demonstrate that the isolate was distinguishable from its relatives based on its D1/D2 domain and ITS region sequences and physiological characteristics. Accordingly, it is concluded that the novel isolate should be assigned to a novel species of the genus Cryptococcus, Cryptococcus taeanensis sp. nov.

Latin diagnosis of Cryptococcus taeanensis Shin & Park sp. nov.


Description of Cryptococcus taeanensis Shin & Park sp. nov.

Cryptococcus taeanensis (tae.an.en’sis. N.L. adj. taeanensis pertaining to Taean, Korea, where the type strain was isolated).

In YM liquid medium after 3 days at 25 °C, cells are spheroïd to ovoid, 4-0–6-3 μm long by 2-6–3-6 μm wide, and occur singly or in pairs. After 1 month at 25 °C, pellicles and sediment are observed. On YM agar after 3 days at 25 °C, the colonies are smooth, butyrous, glistening and cream-coloured with an entire margin. No mycelium or pseudomycelium is formed in Dalmau plate cultures on corn meal agar after 2 weeks.


0.01% or 0.1% cycloheximide. No growth in the presence of 1% acetic acid. Growth occurs in YM broth containing 10% (w/w) NaCl + 5% glucose, but not with 16% (w/w) NaCl + 5% glucose. Weak growth occurs on 50% glucose + yeast extract agar. The production of starch-like compounds is positive. Reaction to Diazonium Blue B is positive. Urease-positive. Growth at 10°C is positive. Reaction to Diazonium Blue B is positive. Growth occurs in YM broth containing 5% (w/w) NaCl + 5% glucose. The major ubiquinone is Q-10. Xylose is present in the cells.

The type strain, 3-12T (= KCTC 17149T = CBS 9742T), was isolated from a saltern in Taean, Korea and deposited in the Korean Collection for Type Cultures, Taejon, Korea.

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References


