Taiwania, **48**(2): 99-105, 2003

Three Species of Yeasts New to Taiwan

Shan-Hua Yang [1] and Pi-Han Wang [1, 2]

(Manuscript received 12 March, 2003; accepted 10 April, 2003)

ABSTRACT: *Cryptococcus flavus* (Saito) Phaff & Fell, *Cr. magnus* (Lodder & Kreger-van Rij) Baptist & Kurtzman, and *Candida azyma* (van der Walt, E. Johannsen & Yarrow) Yarrow & Meyer are on the phylloplane and collected from the mountain sites in Taiwan. They all are new records from Taiwan. Morphological and physiological characteristics of these species are described and illustrated.

KEY WORDS: Yeast, *Cryptococcus flavus*, *Cr. magnus*, *Candida azyma*, Taiwan, Taxonomy.

INTRODUCTION

The genus *Cryptococcus* Vuillemin was established by Kützing in 1833 (Kreger-van Rij, 1984). There were 34 species under this genus that belonged to anamorphic basidiomycetous yeasts and was assigned to Cryptococcaceae (Fell and Statezell-Tallman, 2000). The genus *Candida* Berkhout was established by Berkhout in 1923 (Kreger-van Rij, 1984). There were 163 species under this genus that belonged to anamorphic ascomycetous yeasts was and assigned to Candidaceae (Meyer, Payne and Yarrow, 2000). According to the publication by Council of Agriculture (Wang *et al*., 1999) and Food Industry Research and Development Institute (Liao *et al*., 2000), five *Cryptococcus* spp. and 27 *Candida* spp. have been recorded in Taiwan.

During our studies of yeasts, *Cryptococcus flavus* (Saito) Phaff & Fell, *Cr. magnus* (Lodder & Kreger-van Rij) Baptist & Kurtzman and *Candida azyma* (van der Walt, E. Johannsen & Yarrow) Yarrow & Meyer were on the phylloplane and collected from the mountain sites in Taiwan. They all are recorded for the first time in Taiwan. Morphological and physiological characteristics of these three species were described and illustrated. The cultures of these fungi were preserved in Microbiology Department, Soochow University, Taipei, Taiwan, R. O. C.

MATERIALS AND METHODS

Three typies of plant species, i.e. dominant species, endemic species and indigenous species were selected. Three trees of each plant species were selected and three leaves per plant were taken aseptically at room temperature. Yeasts were isolated by sealing leaf in a sterile plastic bag with 30-40 ml of sterile distilled water per 100 cm^2 of leaf, shaked for 30 min, aliquots of 0.1 ml were spread on plates containing acidified YM agar (yeast extract 0.3%, malt extract 0.3%, peptone 0.5%, glucose 1%, and agar 2%, adjusted to pH3.7 by adding 0.7% of 1M HCl), and incubated at 26°C as described in the literature (Rosa *et al.*, 1995) with modification.

1. Department of Microbiology, Soochow University, Taipei 111, Taiwan.
2. Corresponding author. Tel: +886-2-28819471 ext. 6873; Fax: +886-2-28831193; E-mail: phwang@ mail.scu.

edu.tw

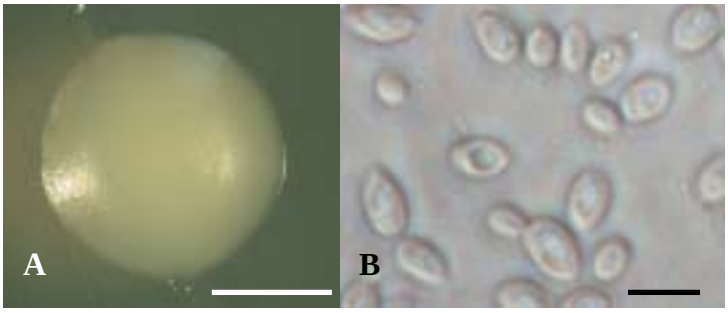

Fig. 1. *Cryptococcus flavus* 42A-7. (A) Colony morphology, 7 days at 26°C in YM agar. Bar = 1 mm. (B) Vegetative cells after 3 days at 26°C in YM agar. Bar = 5 μm. A, DM (Nikon SMZ-10A), B, Ph2 (Nikon ECLIPSE E800).

Single colony for each yeast was selected and grown on another YM agar plate and incubated at 26°C. Yeast morphological characteristics were examined by compound light microscope. When pseudomycelium formation was observed in Dalmau plates, a sterile cover slip was then placed over the center of the streak and another over one of the point inoculation. The cultures were then incubated for 7 days at room temperature and examined microscopically for the formation of filaments along the edges of the streak, both under and around the cover slip (Yarrow, 2000).

Physiological tests were performed by Biolog yeast identification system (Biolog's MicroLog™ 3.0, Biolog, USA) and BCCM™/Allev 2.00 yeast identification system (Robert *et al*., 1997). The results were confirmed to species level according to the original description and keys of Fell and Statezell-Tallman (2000).

TAXONOMY

Cryptococcus flavus (Saito) Phaff & Fell, The Yeast, 1088-1145, 1970.

Growth on YM agar: After 7 days at 26°C, colonies were brownish-yellow, semiglossy and nearly smooth, with a butyrous texture and entire margine (Fig. 1A). A thin ring and a little sediment began to form. After one month a ring and a sediment were present. After 3 days at 26°C, the cells were spherical, ovoid or ellipsoid, single, 3.0-4.0 × 3.0-6.0 μm (Fig. 1B).

Dalmau plate culture on Corn Meal Agar: No pseudomycelium and true mycelium were present.

Physiology: Table 1.

Habitat: On leaves (*Ageratum honstonianum* Mill.)

Specimen studied: Taiwan, ILAN: Fu-Shan research station. Oct 19, 2001. Isolation number *F42A-7*.

Comments: We found that the reaction of L-sorbose was weak rather than negative and the reaction of inulin was negative rather than weak as reported in the original description (Fell and Statezell-Tallman, 2000).

Table 1. Physiological characteristics of *Cryptococcus flavus*, *Cr. magnus* and *Candida azyma* isolated from Taiwan.

Species	1	2	3
Oxidation test			
Acetic acid	/	/	/
Formic acid	-	/	-
Propionic acid	/	/	-
Succinic acid	+	+	/
Methyl succinate	-	-	-
L-aspartic acid	+	+	-
L-glutamic acid	/	+	/
L-proline	/	/	/
D-gluconic acid	+	+	+
Dextrin	/	+	/
Inulin	-	-	-
Cellobiose	+	+	-
Gentiobiose	+	+	-
Maltose	+	+	+
Maltotriose	+	+	-
D-melezitose	+	+	+
D-melibiose	/	/	-
Palatinose	+	+	+
D-raffinose	/	+	-
Stachyose	+	+	-
Sucrose	+	+	+
D-trehalose	+	+	+
Turanose	+	+	+
N-acetyl-D-glucosamine	/	+	-
α-D-glucose	+	+	+
D-galactose	+	+	+
D-psicose	-	+	+
L-sorbose	-	/	+
Salicin	/	+	-
D-mannitol	/	+	+
D-sorbitol	/	+	+
D-arabitol	-	/	+
Xylitol	/	/	+
Glycerol	-	/	+
Tween 80	-	-	+
Assimilation test			
Fumaric acid	+	/	+
L-malic acid	+	+	+
Methyl succinate	-	-	/
Bromo succinic acid	/	+	/
L-glutamic acid	/	/	/
γ-amino butyric acid	/	/	+
α-keto-glutanic acid	/	/	+
2-keto-D-guconic acid	/	+	+
D-gluconic acid	/	+	/
Dextrin	/	+	/
Inulin	-	/	-
Cellobiose	+	+	-

Species	1	2	3
Gentiobiose	+	+	-
Maltose	/	+	+
Maltotriose	/	+	-
D-melezitose	/	+	+
D-melibiose	/	/	-
Palatinose	+	+	+
D-raffinose	+	+	-
Stachyose	+	+	-
Sucrose	+	+	+
D-trehalose	+	+	+
Turanose	+	+	+
N-acetyl-D-glucosamine	/	+	-
D-glucosamine	/	+	-
α-D-glucose	+	+	+
D-galactose	+	/	+
D- psicose	/	/	+
L- rhamnose	/	/	-
L-sorbose	/	/	+
α-methyl-D-glucoside	/	/	+
β-methyl-D-glucosidase	+	+	-
Amygdalin	/	-	+
Arbutin	/	+	-
Salicin	/	+	-
Maltitol	+	+	+
D- mannitol	/	+	+
D- sorbitol	/	+	+
Adonitol	/	/	+
D-arabitol	/	/	/
Xylitol	+	/	+
i-erythritol	+	-	-
Glycerol	-	/	+
Tween 80	/	/	/
L-arabinose	+	+	/
D- arabinose	/	/	/
D-ribose	+	+	-
D-xylose	+	+	+
Methyl succinate+D-xylose	/	/	+
N-acetyl-L-glutamic acid+ D-xylose	/	+	/
Quinic acid+D-xylose	/	+	+
D-glucuronic acid+D-xylose	+	+	+
Dextrin+ D-xylose	+	+	+
α-D-lactose+D-xylose	+	+	/
D-melibiose+D-xylose	+	+	+
D-glactose+D-xylose	+	+	+
m-inositol+ D-xylose	+	+	+
1,2-propanediol+ D-xylose	/	+	+
acetoin+ D-xylose	/	+	+
Growth at 37℃	-	-	-

Species: 1. *Cryptococcus flavus*. 2. *Cryptococcus magnus*. 3. *Candida azyma*. Based on Biolog YT MicroPlate instructions, all reactions optically resembling the negative control wells are scored as "negative" (-) and all wells with a noticeable increase in absorbance at 590 nm are scored as "positive" (+). Well with an extremely slight increase in absorbance at 590 nm are scored as "borderline" (/).

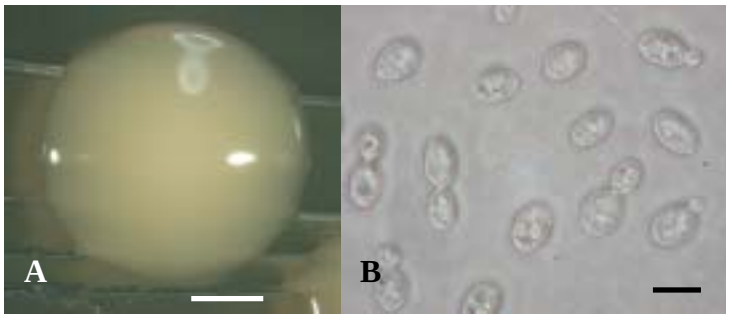

Fig. 2. *Cryptococcus magnus* HG1-1. (A). Colony morphology, 7 days at 26°C in YM agar. Bar = 1 mm. (B). Vegetative cells after 3 days at 26°C in YM agar. Bar = 5 μm. A, DM (Nikon SMZ-10A), B, Ph2 (Nikon ECLIPSE E800).

Cryptococcus magnus (Lodder & Kreger-van Rij) Baptist & Kurtzman, Mycologia 68: 1195-1203, 1976.

Growth on YM agar: After 7 days at 26°C, colonies were cream-colored, the surface was highly glossy, smooth and the texture slimy. The margin was entire. (Fig. 2A). A thin ring and a little sediment began to form. After one month a moderate ring and a little sediment were present. After 3 days at 26°C, the cells were ovoid, single or in pairs, 4.0-5.0 × 7.0-9.0 μm (Fig. 2B).

Dalmau plate culture on Corn Meal Agar: No pseudomycelium and true mycelium were present.

Physiology: Table 1.

Habitat: On leaves (*Hibiscus rosa-sinensis*)

Specimen studied: Taiwan, TAICHUNG: Dadu-shan, Tunghai University. Feb 6, 2001. Isolation number *HG1-1*.

Comments: We found that the reactions of D-glucosamine and N-acetyl-D-glucosamine were positive rather than negative, the reactions of inulin and melibiose were weak rather than negative as reported in the original description (Fell and Statezell-Tallman, 2000).

Candida azyma (van der Walt, E. Johannsen & Yarrow) Yarrow & Meyer, Int. J. Syst. Bacteriol. 28: 611-615, 1978.

Growth on YM agar: After 7 days at 26°C, colonies were white, smooth and semiglossy. The margin was entire (Fig. 3A). After 3 days at 26°C, the cells were ovoid to long ovoid, single or in pairs, 2.0-3.0 × 3.0-6.0 μm (Fig. 3B).

Dalmau plate culture on Corn Meal Agar: No pseudomycelium and true mycelium were present.

Physiology: Table 1.

Habitat: On leaves (*Cycbalannopsis championii*)

Specimen studied: Taiwan, PINDUNG: Nanjen-shan Long-Term Ecological Research Network site. July 29, 2001. Isolation number *ToF1-1*.

Comments: Results from our study were in good agreement with the data as reported in the original description (Meyer, Payne and Yarrow, 2000).

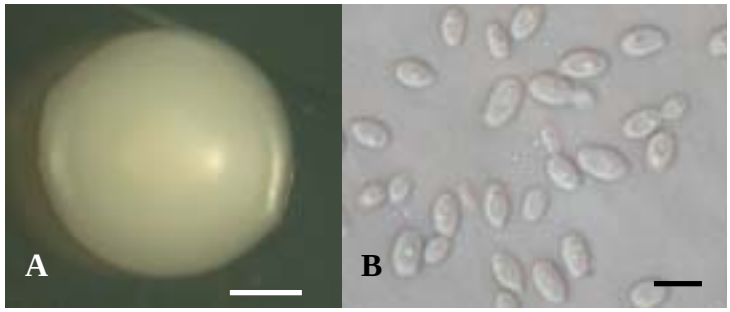

Fig. 3. *Candida azyma* ToF1-1. (A) Colony morphology, 7 days at 26°C in YM agar. Bar = 1 mm. (B) Vegetative cells after 3 days at 26°C in YM agar. Bar = 5 μm. A, DM (Nikon SMZ-10A), B, Ph2 (Nikon ECLIPSE E800).

ACKNOWLEDGEMENTS

This study was supported by the National Science Council, R.O.C., grant number NSC90-2311-B-031-003.

LITERATURE CITED

Baptist, J. N. and C. P. Kurtzman. 1976. Comparative enzyme patterns in *Cryptococcus laurentii* and its taxonomic varieties. Mycologia **68**: 1195-1203.

Fell, J. W. and A. Statzell-Tallman. 2000. *Cryptococcus* Vuillemin. In: Kurtzman, C. P. and J. W. Fell. (eds.). The Yeasts: A Taxonomic Study. Fourth revised and enlarged edition. Elsevier, Amsterdam. pp. 742-767.

Kreger-van Rij, N. J. W. 1984. Systems of classification of the yeasts. In: Kreger-van Rij, N. J. W. (ed.). The Yeasts: A Taxonomic Study. Third revised and enlarged edition. Elsevier. Amsterdam. pp. 2-13.

Liao, C.-C., G.-F. Yuan, F.-L. Lee and B.-C. Wang. 2000. Catalogue of fungi: yeast, filamentous fungi, mushroom. First edition. pp. 1-56. Food Industry Research and Development Institute. Hsinchu, Taiwan.

Meyer, S. A., R. W. Payne and D. Yarrow. 2000. *Candida* Berkhout. In: Kurtzman, C. P. and J. W. Fell (eds.). The Yeasts: A Taxonomic Study. Fourth revised and enlarged edition. Elsevier, Amsterdam. pp. 454-573.

Phaff, H. J. and J. W. Fell. 1970. *Cryptococcus* Kützing emend. Phaff et Spencer. In: Lodder, J. (ed.). The Yeast: A Taxonomic Study. 2nd edition. North-Holland, Amsterdam. pp. 1088-1145.

Robert, V., P. Evrard and G. L. Hennebert. 1997. BCCM ™/Allev 2.00 an automated system for the identification of yeasts. Mycotaxon **64**: 455-463.

Rosa, C. A., P. B. Morais, S. R. Santos, P. R. Peres Neto, L. C. Mendonca-Hagler and A. N. Hagler. 1995. Yeast communities associated with different plant resources in sandy coastal plains of southeastern Brazil. Mycol. Res. **99**: 1047-1054.

Wang, Y.-Z., S.-H. Wu, Y.-N. Chou, T.-T. Chang, K.-Y. Chen, S.-F. Chen, J.-L. Chen, S.-S. Tzean, C.-H. Liu, W.-H. Hsieh, H.-J. Hsieh, C.-H. Chung, and C.-Y. Chien. 1999. List of the fungi in Taiwan. The Council of Agriculture of the Executive Yuan of the R.O.C. 289pp.

Yarrow, D. 2000. Methods for the isolation, maintenance and identification of yeasts. In:

Kurtzman, C. P. and J. W. Fell. (eds.). The Yeasts: A Taxonomic Study. Fourth revised and enlarged edition. Elsevier, Amsterdam. pp. 77-100.

Yarrow, D. and S. A. Meyer. 1978. Proposal for amendment of the diagnosis of the genus *Candida* Berhout nom. cons. Int. J. Syst. Bacteriol. **28**: 611-615.

三種台灣新紀錄種酵母菌

楊姍樺[1]、汪碧涵[1, 2]

(收稿日期：2003 年 3 月 12 日；接受日期：2003 年 4 月 10 日)

摘　　要

本文報導由台灣山區的樹葉上分離得到的兩種隱球菌 (*Cryptococcus* Vuillemin) 屬酵母 *Cryptococcus flavus* (Saito) Phaff & Fell 與 *Cr. Magnus* (Lodder & Kreger-van Rij) Baptist & Kurtzman 以及一種念珠菌 (*Candida* Berkhout) 屬酵母 *Candida azyma* (van der Walt, E. Johannsen & Yarrow) Yarrow & Meyer，皆為台灣新紀錄種，並提供形態和生理上的特徵。所檢查的菌株保存於東吳大學微生物學系。

關鍵詞：酵母菌、*Cryptococcus flavus*、*Cr. magnus*、*Candida azyma*、台灣、分類學。

1.東吳大學微生物系研究所，台北市 111，台灣，中華民國。
2.通信作者。Tel: +886-2-28819471 ext. 6873; Fax: +886-2-28831193; E-mail: phwang@mail.scu.edu.tw