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Rhodotorula calyptogenae, a new record yeast for Taiwan

Chih-Jen Tien[1], Ching-Wen Chang[2], and Pi-Han Wang[1*]

1. Department of Life Sciences, Tunghai University, 181 Taichung Kan Rd., Section 3, Taichung, 40704, Taiwan
2. Department of Microbiology, SooChow University, 70 Linhsi Rd., Shihlin, Taipei, 11102, Taiwan

ABSTRACT

The yeast, *Rhodotorula calyptogenae* Nagahama, Hamamoto, Nakase & Horikoshi is a new record for Taiwan. Its morphological and physiological characteristics are described and illustrated.

Keywords: taxonomy, yeast.

Introduction

Rhodotorula Harrison is a member of the anamorphic basidiomycetous yeasts, occurring naturally in air, clinical, soil, and terrestrial and marine plants and animals (Fell and Statzell-Tallman, 2000). According to Fell and Statzell-Tallman (2000), the genus is diagnosed by spheroidal, ovoidal, or elongate shape of cell. *Rhodotorula* reproduce by multilateral or polar budding. Pseudo- or true hyphae may develop. Ballistoconidia are not formed. Some species of the genus synthesize red or yellow pigments in malt agar cultures. Most, but not all, species lack the ability to assimilated inositol. When inositol is utilized, D-glucuronate is not assimilated. Starch-like substances are not synthesized by any species. Fermentative ability is lacking. Diazonium blue B reaction and presence of urease are positive. Coenzyme Q-9 and Q-10 are present. Xylose is lacking in cell hydrolyzates. Fell *et al.* (2000) studied basidiomycetous yeasts based on the D1/D2 region of 26S rDNA sequences, and classified *Rhodotorula* into two clades, *Sporidiobolus* and *Erythrobasidium*.

Rhodotorula calyptogenae Nagahama, Hamamoto, Nakase & Horikoshi (2003) is a yeast species isolated initially from a giant white clam, *Calyptogena* sp. at 1,156 m deep on the sea floor of Sagami Bay, Japan (Nagahama *et al.*, 2003). The species belongs to the *Erythrobasidium* clade, which included 19 species (Fell *et al.*, 2000). *R. calyptogenae* formed a phylogenetically coherent group with *R. laryngis*, *R. minuta*, *R. pallida*, *R. slooffiae*, *R. benthica*, and *R. lysiniphila* (Nagahama *et al.*, 2003).

Rhodotorula strains from Taiwan were collected from various habitats, including sea water (Chang and Wang, 2002), air, and clinical samples (Chen *et al*., 1998; Hsueh *et al*., 2003).

* Corresponding author. Phone: 886-4-23590121 ext. 32423, Fax: 886-4-23590296, E-mail: phwang@thu.edu.tw.

There were six *Rhodotorula* species reported in Taiwan, including *R. aurantiaca*, *R. graminis*, *R. marina*, *R. minuta*, *R. mucilaginosa*, and *R. slooffiae* (Chang and Wang, 2002; Chen *et al*., 1998; Hsueh *et al*., 2003), those cultures were preserved in the Bioresource Collection and Research Center (BCRC), Food Industry Research and Development Institute, Hsinchu, Taiwan.

We recorded *R. calyptogenae* strain 4107 for the first time in Taiwan, and described its morphological and physiological characteristics. The culture was maintained in BCRC as BCRC 23153.

Materials and Methods

Water samples were collected from coast. These samples (1,000 mL) were concentrated to 1 mL by centrifuging at 1,000 x*g* for 5 min. Concentrated samples were plated onto acidified (pH 4.4) YM agar (yeast extract-malt extract agar, yeast extract 3 g, malt extract 3 g, peptone 5 g, glucose 10 g, agar 18 g, distilled water 1,000 mL) plates containing 40 mg/L chloramphenicol (Yarrow, 2000) and incubated 3 to 5 days at 25ºC. Yeast strains were purified by streaking (Yarrow, 2000).

Isolated yeasts were grown on YM agar and incubated at 25 ºC. Dalmau plate culture was performed on corn meal agar (Yarrow, 2000). Morphological characteristics were observed by using light microscope and scanning electron microscope was performed by Instrumentation Center of National Taiwan University. Physiological characteristics were tested with the Biolog yeast identification kits (Biolog, California, USA). Results of all common Biolog plate tests agree with reaction responses of traditional methods (Kreger van Rij, 1984; Barnett *et al*., 1990 Praphailong *et al*., 1997).

Genomic DNA was extracted by the CTAB method adapted from Doyle and Doyle (1990) and described by Wang *et al.* (2003). The internal transcribed spacer (ITS) region of ribosomal DNA (rDNA) was amplified by PCR (White *et al*., 1990) with primers ITS1 and ITS4 (White *et al*., 1990). The D1/D2 domain of large subunit (LSU) rDNA was amplified by primer F63 (5’ -GCA TAT CAA TAA GCG GAG GAA AAG- 3’) and LR3 (5’ -GGT CCG TGT TTC AAG AAC G- 3’) (Fell *et al*., 2000). PCR products were sequenced (Mission Biotech, Taipei, Taiwan) and the sequences were checked against GenBank database using BLAST.

Results and Discussion

The D1/D2 domain sequence of strain 4107 (EU669877), showed only one base (628/629 bp) difference from *R. calyptogenae* (AB025996) (Nagahama *et al.*, 2003). It had 18 bases (611/629 bp) differerences from *R. lysiniphila* (AB078501) (Nagahama *et al.*, 2003), the nearest related species to *R. calyptogenae* (Nagahama *et al.*, 2003). The ITS region sequence of strain 4107 (EU669878) had 3 bases (568/571 bp) differ from *R. calyptogenae* (AB025996) and 23 bases (611/629 bp) differ from *R. lysiniphila* (AB078501) (Nagahama *et al*., 2003).

The sequence variability of a yeast species can be illustrated by the number of base changes between isolates. How many sequence variation of rDNA D1/D2 domain and ITS region within a species are acceptable? Between the isolates of the *Rodotorula* species, 3 nucleo-

tide differences of D1/D2 domain and ITS region are not uncommon (Scorzetti *et al*., 2002).

In general, variation of D1/D2 domain within ascomycetous yeasts species show no more than 0-3 nucleotide difference (0–0.3%); strains showing 6 or more nucleotide difference are separate species (Kurtzman and Robnett, 1998; Kurtzman and Piškur, 2005). Kurtzman and Piškur (2005) commented that D1/D2 database could be used in quickly classify most known species, and discover new species. With the concept, we compared strain 4107 to the type strain of *R. calyptogenae* in extremely way, only 1 base substitution in D1/D2, and 3 bases substitution in ITS. The nucleotide difference was not considered enough to exclude strain 4107 from *R. calyptogenae*.

Taxonomy

Rhodotorula calyptogenae Nagahama, Hamamoto, Nakase & Horikoshi, 2003. IJSEM 53: 897–903.

Morphology. Growth on YM agar: After 7 days at 25ºC, streak culture was light-orange, glistening, soft, and had an entire margin (Fig. 1A). Growth in YM broth: After 3 days at 25ºC, cells were oval and ellipsoidal, 3.5–5.5 × 4.5–7.0 μm, single or in pair (Fig. 1B & Fig. 1C). Dalmau plate culture on corn meal agar, no branching hyphae or pseudohyphae were formed.

Physiological characteristics. See Table 1. In this study, we tested 94 characters of strain 4107 using the Biolog YT MicroPlate™. Thirty one of these characters were comparable to characteristics described by Nagahama *et al.* (2003). Among those tests, L-sorbose and lactose stimulate a weak reaction in type strain of *R. calyptogenae* (Nagahama *et al.*, 2003). However, strain 4107 showed negative reactions at both tests.

Sequences. Sequence analysis of D1/D2 domain of 26S rDNA for strain 4107 (EU669877) showed substantial homogeneity (628/629 bp) with sequence (AB025996) of type strain *R. calyptogenae* (= JCM 10899^T = CBS 9125^T). Sequence (EU669878) of rDNA ITS region of strain 4107 also showed greatest similarity (568/571 bp) with sequence (AB025996) of *R. calyptogenae.*

Specimen examined. Specimen BCRC 23153 was isolated from sea water of Golden Coast (N22º55'52", E120º10'33") near Tainan City, Taiwan on 16 July 1999. Isolation number 4107 (= BCRC 23153).

Based on the physiological, biochemical, and genetic characters, we identified strain 4107 as *R. calyptogenae*. The strain showed intraspecies variations of physiological and genetic

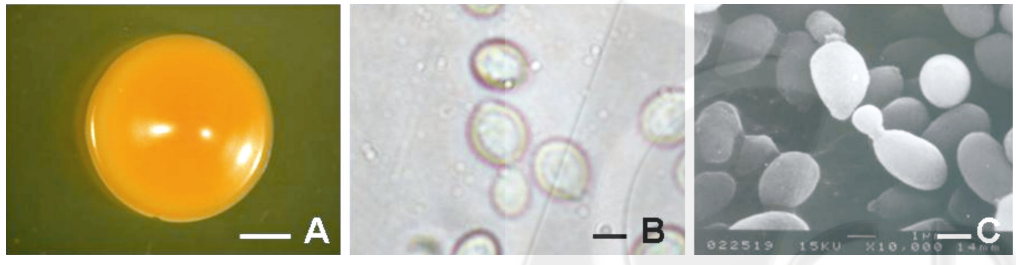

Fig. 1. *Rhodotorula calyptogenae* strain 4107. A. Colony morphology, 7 days at 25°C on YM agar. Bar = 1 mm. B & C. Vegetative cells after 3 days at 25ºC on YM agar, Bar = 1 μm. A, dissecting microscope; B, light microscope; C, SEM.

Table 1. Physiological characteristics of *Rhodotorula calyptogenae* strain 4107[a] and JCM 10899^{T} [b].

	4107	JCM 10899^{T}		4107	JCM 10899^{T}		4107	JCM 10899^{T}
Oxidation test			**Assimilation test**			Maltitol	−	
Acetic acid	+		Fumaric acid	+		D-mannitol	−	+
Formic acid	−		L-malic acid	−		D-sorbitol	+	
Propionic acid	+		Methyl succinate	−		Adonitol	−	
Succinic acid	−		Bromo succinic acid	+	+	D-arabitol	+	
Methyl succinate	+		L-glutamic acid	+		Xylitol	−	
L-aspartic acid	+		γ-amino butyric acid	+		i-erythritol	−	−
L-glutamic acid	−		α-keto-glutanic acid	+		Glycerol	+	+
L-proline	+		2-keto-D-guconic acid	+	+	Tween 80	+	
D-gluconic acid	+		D-gluconic acid	+		L-arabinose	+	+
Dextrin	+		Dextrin	+		D-arabinose	/	−
Inulin	+		Inulin	−	−	D-ribose	/	−
Cellobiose	+		Cellobiose	+	+	D-xylose	+	+
Gentiobiose	−		Gentiobiose	+		Methyl succinate + D-xylose	+	
Maltose	−		Maltose	−	−	N-acetyl-L-glutamic acid + D-xylose	+	
Maltotriose	+		Maltotriose	−		Quinic acid + D-xylose	+	
D-melezitose	−		D-melezitose	+	+	D-glucuronic acid + D-xylose	+	
D-melibiose	−		D-melibiose	−	−	Dextrin + D-xylose	+	
Palatinose	−		Palatinose	−		α-D-lactose + D-xylose	+	
D-raffinose	−		D-raffinose	−	−	D-melibiose + D-xylose	+	
Stachyose	+		Stachyose	−		D-glactose + D-xylose	+	
Sucrose	+		Sucrose	+	+	m-inositol + D-xylose	+	
D-trehalose	−		D-trehalose	+	+	1,2-propanediol + D-xylose	+	
Turanose	−		Turanose	/		Acetoin + D-xylose	+	
N-acetyl-D-glucosamine	−		N-acetyl-D-glucosamine	−		**Additional test**		
α-D-glucose	+		D-glucosamine	/		Lactose	−	+
D-galactose	/		α-D-glucose	+	+	starch	−	−
D-psicose	+		D-galactose	+	+	ethanol	+	+
L-sorbose	−		D- psicose	+		Galactitol	−	−
Salicin	+		L- rhamnose	−	−	inositol	−	−
D-mannitol	−		L-sorbose	−	+	citrate	−	−
D-sorbitol	−		α-methyl-D-glucoside	−	−	0.1% cycloheximide	+	
D-arabitol	−		β-methyl-D-glucosidase	+		Starch formation	−	−
Xylitol	−		Amygdalin	/		Diazonium Blue B	+	+
Glycerol	+		Arbutin	+		Ballistocondida	−	
Tween 80	/		Salicin	+	+	Symmetric ballistocondida	−	

[a] Methods followed Biolog YT MicroPlate™. Reactions were scored based on absorbance at 590 nm: no absorbance was negative –, noticeable absorbance was positive +, and slight increase absorbance was borderline /.

[b] Data are from Nagahama *et al.*(2003).

characteristics from type strain. Since *R. calyptogenae* was named, it has been considered as a deep-sea species. Strain 4107 was the first strain to be isolated and identified in Taiwan. Our finding demonstrated that the species can also be found in the surface layer.

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臺灣新紀錄種巨白蛤紅酵母

田志仁[1]　張瀞文[2]　汪碧涵[1]

1. 東海大學生命科學系
2. 東吳大學微生物學系

摘　要

本文描述由臺南黃金海岸的海水中分離的到之巨白蛤紅酵母 *Rhodotorula calyptogenae* Nagahama, Hamamoto, Nakase & Horikoshi，為臺灣之新紀錄種。

關鍵詞：*Rhodotorula calyptogenae*、分類學、巨白蛤紅酵母菌、臺灣。