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Four Species of *Pichia* Yeasts New to Taiwan

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ABSTRACT: *Pichia anomala*, *P. guilliermondii*, *P. kluyveri*, and *P. ohmeri* are new records from Taiwan. Morphological and physiological characteristics of these species are described and illustrated.

KEY WORDS: **Yeast, *Pichia anomala*, *P. guilliermondii*, *P. kluyveri*, *P. ohmeri*, Taiwan, Taxonomy.**

INTRODUCTION

The genus *Pichia* established by Hansen in 1904, and named in honor of Professor P. Pichi (Kurtzman and Fell, 2000). There were 91 species under the genus that belonged to Ascomycota and assigned to the Saccharomycetaceae (Kurtzman and Fell, 2000). *Pichia* was a common and dominant genus in terrestrial habitats, and associated with plants and soil (Phaff and Starmer, 1987). Some species of *Pichia* have important use in application. *Pichia pastoris* have been developed into highly successful system for the production of a variety of heterologous proteins (Cereghino and Cregg, 2000). *Pichia anomala*, *P. guilliermondii*, and *P. membranifaciens* inhibit fungal diseases, and can be used as biological control organisms (Petersson and Schnürer, 1995; Masih *et al.*, 2001). Middelbeek *et al.* (1980) have shown that the killer toxin of *Pichia kluyveri* is strongly active against various strains of *Saccharomyces cerevisiae*, *Candida albicans, C. krusei, C. parapsilosis, C. tropicalis* and *Torulopsis glabrata.*

In the clean aquatic regions, *Pichia* spp. were widespread, the commonest species found were *P. kluyveri* and *P. terricola* (Ahearn *et al.*, 1968; Spencer *et al.*, 1974; Hagler *et al.*, 1982).

In 1977, Cheng and Lin first recorded *Pichia hansen*, which was collected from seawater of the west coast, Taiwan. During our studies of yeasts, *Pichia anomala*, *P. kluyveri*, and *P. ohmeri* were collected from Tamshui River, and *P. guilliermondii* were collected from Er-Jen River, Taiwan. They all are recorded for the first time in Taiwan. Morphological and physiological characters of these four species are described and illustrated. The cultures are preserved in Microbiology Department, Soochow University, Taipei, Taiwan, R.O.C., and Culture Collection and Research Center, Food Industry Research and Development Institute, Hsinchu, Taiwan, R.O.C.

MATERIALS AND METHODS

Water samples were collected from Tamshui River and Er-Jen River of Taiwan. Water samples (1,000 mL) were concentrated to 1 mL by centrifugation at 1,000 x*g* for 5 min. The concentrated samples were plated on acidified (pH 4.4) YM agar (yeast extract-malt extract

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agar, yeast extract 3 g, malt extract 3 g, peptone 5 g, glucose 10 g, agar 18 g, distilled water 1,000 mL) plates that contain 40 mg/L chloramphenicol (Kurtzman and Fell, 2000) and incubated 3 to 5 days at 25℃. Yeast isolates were purified by four-way streak-plate method.

Isolated yeasts were grown on YM agar and incubated at 25℃. Varieties morphological characters were observed by compound light microscope and scanning electron microscope (Instrumentation Center, National Taiwan University). Ascospores were observed on McClary's acetate agar (glucose 1 g, KCl 1.8 g, yeast extract 2.5 g, sodium acetate trihydrate 8.2 g, agar 15 g, distilled water 1,000 mL) (Kurtzman and Fell, 2000) and incubated 7 to 10 days at 25℃, dyeing with cotton blue. Pseudohyphae formation was observed in Dalmau plates, a sterile cover slip is placed over the center of the streak and another over one of the point inoculations. The cultures were then incubated for 3 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 (Kurtzman and Fell, 2000). Physiological tests were performed by Biolog yeast identification kits (Biolog, USA). Based on morphological and physiological characters, isolates were confirmed to species level according to the descriptions and keys of Kurtzman and Fell (2000).

TAXONOMY

Pichia anomala (E. C. Hansen) Kurtzman, Antonie van Leeuwenhoek 50: 209-217, 1984.

Growth on YM agar: After 7 days at 25℃: Colony white, butyrous (Fig. 1A). After 3 days at 25℃, cells spheroidal to elongate, 2.0-5.0 × 3.0-6.0 μm, and growing singly, in pairs, or in small clusters (Figs. 1B, C).

Dalmau plate culture on Corn Meal Agar: Pseudohyphae consist of chains of ovoidal or cylindroidal cells.

Formation of ascospores: Strain ST3-1d formed asci that contained 2 to 4 hat-shaped ascospores on McClary's acetate agar after 7 days (Fig. 1D). It should be sporogenous diploid. Kurtzman and Fell (2000) reported that *P. anomala* is heterothallic, but the sporogenous diploid form is usually isolated from nature and diploid cells directly convert to asci and form 1 to 4 hat-shaped ascospores.

Physiology: Table 1.

Habitat: In river water.

Specimen studied: Taiwan. Taipei, Tamshui River, Bailing Bridge. Aug 17, 2000. Isolation number ST3-1d (CCRC 22921).

Pichia guilliermondii Wickerham, J. Bacteriol. 92: 1269, 1966.

Growth on YM agar: After 7 days at 25℃: Colonies smooth and tannish-white in color (Fig. 2A). After 3 days at 25℃, the cells ovoidal to elongate, 1.5-3.0 ×1.5-4.5 μm, single, in pairs or in short clusters, reproduction by budding (Figs. 2B, C).

Dalmau plate culture on Corn Meal Agar: Pseudohyphae show abundant, well-branched bearing whorls of blastoconidia (Fig. 2D).

Formation of ascospores: Strain SE2H-6 was haploid form and did not form asci on McClary's acetate agar after 10 days. Kurtzman and Fell (2000) reported that *P. guilliermondii* is heterothallic and only haploid forms have been isolated.

Physiology: Table 1.

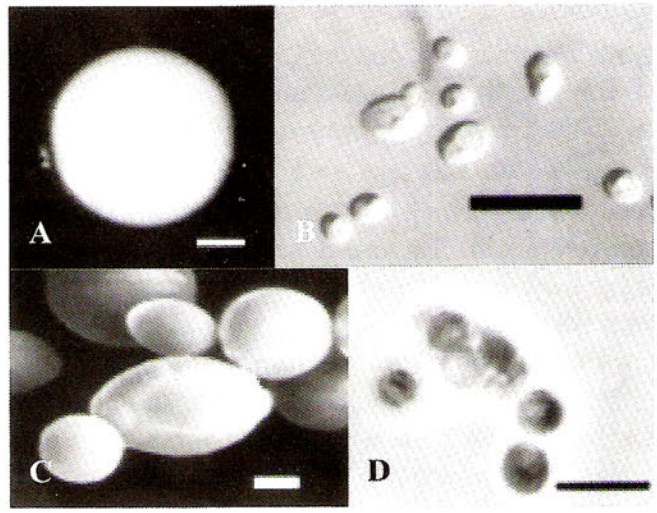

Fig. 1. *Pichia anomala* ST3-1d (CCRC 22921). (A) Colony morphology, 7 days at 25℃ in YM agar. Bar = 1 mm. (B) Vegetative cells after 3 days at 25℃ in YM agar, Bar = 10 μm. (C) Vegetative cells after 3 days at 25℃ in YM agar, Bar = 1 μm. (D) Asci and ascospores after 7 days at 25℃ in McClary's acetate agar. Bar = 10 μm. A, DM (Olympus SZ-ET), B & D, LM (Nikon ECLIPSE E800), C, SEM.

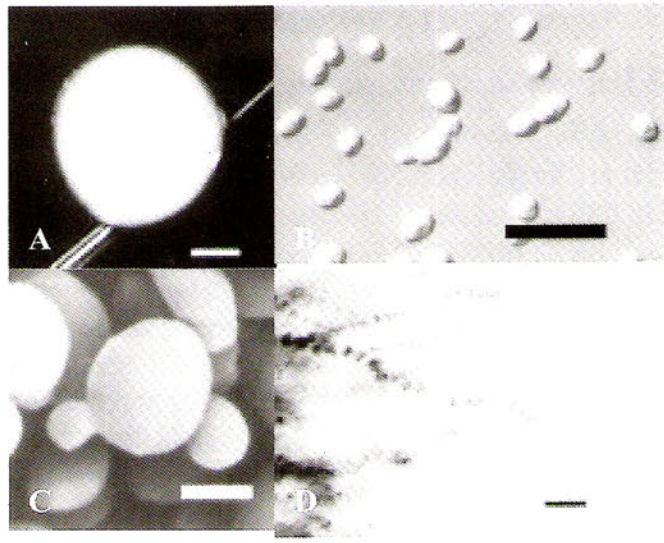

Fig. 2. *Pichia guilliermondii* SE2H-6 (CCRC 22922). (A) Colony morphology, 7 days at 25℃ in YM agar. Bar = 1 mm. (B) Vegetative cells and its budding after 3 days at 25℃ in YM agar. Bar = 10 μm. (C) Vegetative cells with one budding after 3 days at 25℃ in YM agar. Bar = 1 μm. (D) Pseudomycelium, 7 days at 25℃ in CMA agar. Bar = 10 μm. A, DM (Olympus SZ-ET), B & D, LM (Nikon ECLIPSE E800), C, SEM.

Habitat: In river water.

Specimen studied: Taiwan. Kaohsiung, Er-Jen River, Ercengxing Bridge. Sep 13, 2000. Isolation number SE2H-6 (CCRC 22922).

Pichia kluyveri Bedford ex Kudryavtsev, Die Systematik der Hefen. Berlin: Akademie Verlag, 1960.

Growth on YM agar: After 7 days at 25℃: Colonies grayish to cream-colored, dull, soft, winkled (Fig. 3A). After 3 days at 25℃, cells ovoidal to cylindroidal, 2.0-3.5 × 3.0-5.0 μm, single, in pairs and chains, reproduction by budding (Figs. 3B, C).

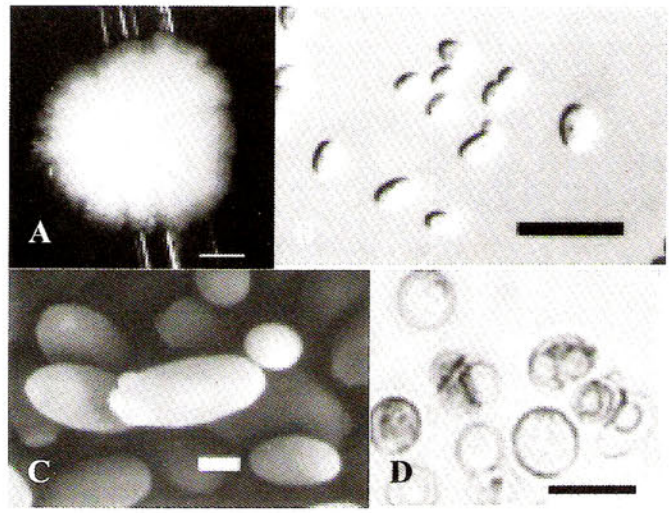

Fig. 3. *Pichia kluyveri* ST3-5 (CCRC 22923). (A) Colony morphology, 7 days at 25℃ in YM agar. Bar = 1 mm. (B) Vegetative cells after 3 days at 25℃ in YM agar. Bar = 10 μm. (C) Vegetative cells after 3 days at 25℃ in YM agar. Bar = 1 μm. (D) Asci and ascospores after 7 days at 25℃ in McClary's acetate agar. Bar = 5 μm. A, DM (Olympus SZ-ET), B & D, LM (Nikon ECLIPSE E800), C, SEM.

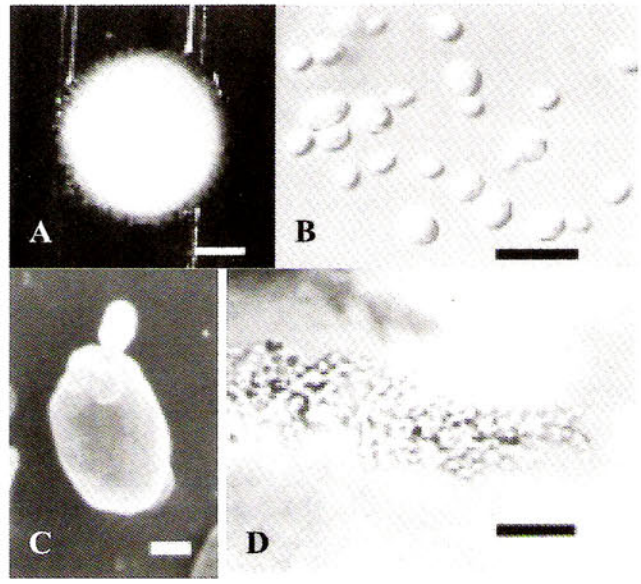

Fig. 4. *Pichia ohmeri* ST5-3 (CCRC 22924). (A) Colony morphology, 7 days at 25℃ in YM agar. Bar = 1 mm. (B) Vegetative cells after 3 days at 25℃ in YM agar. Bar = 10 μm. (C) Vegetative cells after 3 days at 25℃ in YM agar. Bar = 1 μm. (D) Pseudomycelium, 7 days at 25℃ in CMA agar. Bar = 10 μm. A, DM (Olympus SZ-ET), B & D, LM (Nikon ECLIPSE E800), C, SEM.

Dalmau plate culture on Corn Meal Agar: Pseudohyphae consist of chains of ovoidal or cylindroidal cells, sometimes bear small verticals of ovoidal blastoconidia.

Formation of ascospores: Strain ST3-5 was homothallic and formed asci that contained 2 hat-shaped ascospores on McClary's acetate agar after 7 days without conjugation of complementary mating types (Fig. 3D). Kurtzman and Fell (2000) reported that most strains of *P. kluyveri* are heterothallic with 2 to 4 hat-shaped spores in each ascus. But some, including the two-spored strains, appear homothallic (Kurtzman and Fell, 2000).

Table 1. Physiological characteristics of four species of *Pichia* genus isolated from Taiwan river water.

Species	1	2	3	4
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	4
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. *Pichia anomala* (CCRC 22921). 2. *Pichia guilliermondii* (CCRC 22922). 3. *Pichia kluyveri* (CCRC 22923). 4. *Pichia ohmeri* (CCRC 22924). 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" (\).

Physiology: Table 1.

Habitat: In river water.

Specimen studied: Taiwan. Taipei, Tamshui River, Bailing Bridge. Aug 17, 2000. Isolation number ST3-5 (CCRC 22923).

Pichia ohmeri (Etchells & T. A. Bell) Kreger-van Rij, Thesis. University of Leiden, the Netherlands, 1964.

Growth on YM agar: After 7 days at 25℃: Colonies grayish-white, and nearly smooth to finely convoluted. Margins irregularly lobed (Fig. 4A). After 3 days at 25℃, the cells ovoidal to cylindroidal, 2.0-5.0 ×2.5-7.5 μm, single, in pairs and in short chains. Reproduction by budding (Figs. 4B, C).

Dalmau plate culture on Corn Meal Agar: Pseudohyphae consist of chains of ovoidal or cylindroidal cells, and sometimes bear small verticals of ovoidal blastoconidia (Fig. 4D).

Formation of ascospores: Strain ST5-3 did not form asci on McClary's acetate agar after 10 days. It should be haploid form. Kurtzman and Fell (2000) reported that *P. ohmeri* is heterothallic species that normally isolated in the haploid from and require conjugation of complementary mating types before sporulation, diploid strains which form unconjugated asci are infrequency isolated (Kurtzman and Fell, 2000).

Physiology: Table 1.

Habitat: In river water.

Specimen studied: Taiwan. Taipei, Tamshui River, Chuchih Dam. Aug 17, 2000. Isolation number ST5-3. (CCRC 22924)

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四種台灣新紀錄的畢赤酵母菌

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摘　　要

本文報導四種由台灣河水中分離到的畢赤酵母 (*Pichia* E. C. Hansen emend. Kurtzman)，*Pichia anomala* (CCRC 22921)、*P. guilliermondii* (CCRC 22922)、*P. kluyveri* (CCRC 22923) 與 *P. ohmeri* (CCRC 22924)，皆為台灣之新紀錄種。

關鍵詞：酵母菌、*Pichia anomala*、*P. guilliermondii*、*P. kluyveri*、*P. ohmeri*、台灣、分類學。

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