TWO NEW SPECIES OF THE GENUS *CANDIDA* FROM WILD GRAPES

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Two new species of the genus *Candida* from wild grapes (*Vitis coignetiae*) in central Japan are described. The new taxa are named *Candida agrestis* and *Candida montana*.

In a study of the yeast flora of wild grapes (*Vitis coignetiae*) in central Japan, two yeasts were found to be previously undescribed representatives of the genus *Candida*.

MATERIALS AND METHODS

Yeast cultures. Five yeast strains, No. 611, No. 616, No. 624, No. 865, and No. 868 taken from wild grapes in the Hirose region of Yamanashi Prefecture were examined.

Identification. The descriptions of the species are based on the standard methods adopted for yeast taxonomy (1). YM broth (5 g peptone, 3 g malt extract, 3 g yeast extract, and 10 g glucose in 1,000 ml distilled water) was used for liquid cultures. Fifteen grams of agar was added to YM broth in solid medium.

Biochemical characters. The colour reaction with Diazonium Blue B reagent was tested on 3-week-old cultures on YM agar as described by VAN DER WALT and HOPSE-HAVU (2). Vitamin requirements were determined by the method of GOTO (3). Urease activity was determined according to the method of SEELIGER (4). The DNA base composition (GC content) was determined according to the procedure of NAKASE and KOMAGATA (5). The ubiquinone system was determined by the method of YAMADA and KONDO (6). Extracellular DNase was measured by the modified procedure of SEN and KOMAGATA (7) of CAZIN et al. (8).

RESULTS AND DISCUSSION

Description of Candida agrestis Goto et Oguri sp. nov.

Cultura in YM (peptonum-extractum malto-extractum faecis jus): Post 3 dies

Fig. 1. Vegetative cells of *Candida agrestis* No. 611 in YM medium after 3 days at 25°.

Fig. 2. Primitive pseudomycelium of *Candida agrestis* No. 611. Slide culture on potato glucose agar for 7 days at 25°.

Fig. 3. Vegetative cells of *Candida montana* No. 865 in YM medium after 3 days at 25°.

Fig. 4. Primitive pseudomycelium of *Candida montana* No. 865. Slide culture on potato glucose agar for 7 days at 25°.

Bar=10 μ m.

ad 25° cellulae ovoideae aut globosae, 3–5 $\times$ 4–6.5 μ m, singulae aut catenatae breves; sedimentum tenue formantur. Cultura in striis agaro YM; Post unum mensem ad 20° color cremeus, pagina nitrida et laevis, margo integer. Pseudomycelium primitivum formantur.

Ascosporae, ballistosporae et teliosporae nullae.

Fermentat glucosum. Assimilat glucosum, sucrosam, cellobiosum, trehalosum, raffinosem, melezitosem (exiguum), ethanolum, ribitolum (exiguum), D-mannitolum, D-glucitolum, α -methyl-D-glucosidum (valde exiguum), acidum lacti-

cum (exigue) et acidum succinicum (exigue).

Kalium nitricum non assimilatur, amylum non formatur, ureum non finditur. As crescentiam vitaminæ necessariae sunt. In 37° non crescit. Proportio molaris guanini+cytosini in acido deoxyribonucleico 37.8 mol%.

Habitat in baccis *Vitis coignetiarum*.

Holotypus: Stirps 611 ex baccis *Vitis coignetiarum*, Hirose, Prov. Yamanashi, Japonia, leg. S. Goto, 3. X. 1979 isolata est. In collectione culturarum, "The Institute of Enology and Viticulture, Yamanashi University, Kofu", sub RIFY 4611 deposita est.

Growth in YM broth: After 3 days at 25°, cells are oval to globose, 3–5 × 4–6.5 µm, single, in pairs and in short chains (Fig. 1). A thin pellicle is formed.

Growth on YM agar: After one month at 20°, the streak culture is cream-colored, glistening, smooth, entire at the margin.

Slide culture on potato-glucose agar: Primitive pseudomycelium is formed (Fig. 2).

Sporulation: No ascospores, ballistospores and teliospores are formed.

Fermentation: Only glucose is fermentation; no other sugars are fermented.

Assimilation of carbon sources:

Glucose	+	D-Ribose	—
Galactose	—	L-Rhamnose	—
L-Sorbose	—	Ethanol	+
Sucrose	+	Glycerol	—
Maltose	—	Erythritol	—
Cellobiose	+	Ribitol	+ weak
Trehalose	+	Galactitol	—
Lactose	—	D-Mannitol	+
Melibiose	—	D-Glucitol	+
Raffinose	+	α-Methyl-D-	
Melezitose	+ weak	glucoside	+ very weak
Inulin	—	Salicin	—
Starch	—	DL-Lactic acid	+ weak
D-Xylose	—	Succinic acid	+ weak
L-Arabinose	—	Citric acid	—
D-Arabinose	—	Inositol	—

Assimilation of potassium nitrate: Negative.

Growth in vitamin-free medium: Negative. Thiamine and pyridoxine are required for growth.

Sodium chloride tolerance: 3% (w/v).

Growth temperature: Positive at 34°, negative at 37°.

Production of starch-like compounds: Negative.

Activities of urease and extracellular DNase: Negative.

GC content of DNA: 37.8 mol %.

Coenzyme Q system: Q 7.

Diazonium Blue B reaction: Negative.

Strains examined: Three strains, No. 611 (holotype), No. 616 and No. 624, were obtained from the berries of *Vitis coignetiae*, collected at Hirose, Yamanashi Prefecture, October 13, 1979, by S. Goto. The strains 611, 616 and 624 have been deposited in the culture collection of the Institute of Enology and Viticulture, Yamanashi University, Kofu, as RIFY 4611 (No. 611), 4616 (No. 616) and 4624 (No. 624), and in the Centraalbureau voor Schimmelcultures, Delft, as CBS 8055 (RIFY 4611).

Description of Candida montana Goto et Oguri sp. nov.

Cultura in YM (peptonum-extractum malto-extractum faecis jus): Post 3 dies ad 25° cellulae globosae aut ovoideae, $2-3 \times 2-4.5 \mu\text{m}$, singulae, binae vel catenatae breves; mitivum. Sedimentum formatur. Cultura in striis agarō YM: ad 20°, post unum mensem ad 20° color cremeus, pagina laevis, margo integer. Pseudomycelium primitivum formatur.

Ascosporae, ballistosporae et teliosporae nullae.

Fermentat glucosum (valde exigue). Assimilat glucosum, sucrosus, cellobiosum, raffinose (valde exigue), meleztosum (valde exigue), L-rhamnosum, ethanolum, glycerolum, D-mannitolum, D-glucitolum, alpha-methyl-D-glucosidum (valde exigue), salicinum, acidum lacticum, acidum succinicum et acidum citricum.

Kalium nitricum non assimilatur, amylum non formatur, ureum non finditur. Ad crescentiam vitaminarum externarum non necessariae sunt. In 41° non crescit. Proportio molaris guanini+cytosini in acido deoxyribonucleico 35.6 mol %.

Habitat in baccis *Vitis coignetiarum*.

Holotypus: Stirps 865 ex baccis *Vitis coignetiarum*, Hirose, Prov. Yamanashi, Japonia, leg. S. Goto, 27. IX. 1978 isolata est. In collectione culturarum, "The Institute of Enology and Viticulture, Yamanashi University, Kofu", sub RIFY deposita est.

Growth in YM: After 3 days at 25°, cells are globose to oval, $2-3 \times 2-4.5 \mu\text{m}$, single, in pairs or in short chains (Fig. 3). A sediment is formed.

Growth on YM agar: After one month at 20°, the streak culture is cream-colored, smooth, entire at the margin.

Slide culture on potato-glucose agar: Primitive pseudomycelium is formed (Fig. 4).

Sporulation: No ascospores, ballistospores, and teliospores are formed.

Fermentation: Very weak positive, glucose only, no other sugars are fermented.

Assimilation of carbon sources:

Glucose	+	D-Ribose	—
Galactose	—	L-Rhamnose	+

L-Sorbose	—	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	—	Erythritol	—
Cellobiose	+	Ribitol	—
Trehalose	—	Galactitol	—
Lactose	—	D-Mannitol	+
Melibiose	—	D-Glucitol	+
Raffinose	+very weak	α -Methyl-D-	
Melezitose	+very weak	glucoside	+very weak
Inulin	—	Salicin	+
Starch	—	DL-Lactic acid	+
D-Xylose	—	Succinic acid	+
L-Arabinose	—	Citric acid	+
D-Arabinose	—	Inositol	—

Assimilation of potassium nitrate: Negative.

Growth in vitamin-free medium: Very weak growth.

Vitamin stimulating growth: Biotin, thiamine, and pyridoxine.

Sodium chloride tolerance: 6% (w/v).

Growth at 37°: Positive; at 41°: Negative.

Production of starch-like compounds: Negative.

Activities of urease and extracellular DNase: Negative.

GC content of DNA: 35.6 mol %.

Coenzyme Q system: Q 7.

Diazonium Blue B reaction: Negative.

Strains examined: Two strains, No. 865 (holotype) and No. 868, were isolated from *Vitis coignetiae* at Hirose, Yamanashi Prefecture, September 27, 1978 by S. Goto. The strains 865 and 868 have been deposited in the Culture Collection of the Institute of Enology and Viticulture, Yamanashi University, Kofu, as RIFY 4865 (No. 865) and 4968 (No. 868), and in the Centraalbureau voor Schimmelcultures, Delft, as CBS 8057 (RIFY 4865).

The two yeasts *C. agrestis* and *C. montana* from wild grapes (*Vitis coignetiae*) were classified in the genus *Candida* because they do not form ascospores, ballistospores, and teliospores and do not produce carotenoid pigments.

C. agrestis and *C. montana* ferment only glucose. The assimilation patterns of the carbon compounds of the two yeasts are unlike those of any of the known nitrate-negative, the low GC content (below 40 mol %), and urease- and DNase-negative *Candida* species. The two species are in the delimitation of the genus *Torulopsis* Berless. YARROW and MEYER (9) proposed an amendment of the diagnosis of the genus *Candida* Berkhout to include non-hyphal species. In accordance with the amended description, these yeasts are classified in the genus *Candida*.

Distribution of the yeasts on wild grapes will be reported in a separate paper (10).

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