

## Taxonomical study of yeasts and yeast-like microorganisms isolated from the denitrification unit biocenosis

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Sláviková E., Grabińska-Łoniewska A.: *Taxonomical study of yeasts and yeast-like microorganisms isolated from the denitrification unit biocenosis*. Acta Mycol. 23(2): 81-87, 1987 (1990).

A set of 8 strains of yeasts and yeast-like microorganisms was isolated from the denitrification unit biocenosis fed with a synthetic medium containing methanol as a carbon source. These strains were identified as *Candida boidinii*, *C. maltosa*, *Rhodotorula rubra* and *Trichosporon cutaneum*.

### INTRODUCTION

In our earlier paper (Sláviková and Grabińska-Łoniewska, 1988 (1989) we described morphological and physiological characters of 13 strains of yeasts and yeast-like microorganisms populating denitrification unit biocenosis grown in continuous system in medium with acetic acid as carbon source. We classified them as *Candida famata*, *C. lambica*, *C. boidinii*, *C. inconspicua*, *C. tropicalis*, *Geotrichum candidum* and *Hansenula californica*. Basing on the results of physiological investigations we determined that those organisms did not take part in the nitrate removal process. The major growth substrate for them were compounds released from autolyzed denitrifying bacterial cells not a synthetic mineral medium used as a feed in a course of denitrification process.

This paper considers the identification of yeasts and yeast-like microorganisms isolated from the sludge fed with medium containing methanol as a carbon source.

## MATERIALS AND METHODS

The yeasts strains were isolated in the period of X.1983-V.1984 from the denitrifying sludge derived from the laboratory unit, situated in Warsaw Technical University. Reactor UASB-type was feeding with a synthetic medium contained (in mg l<sup>-1</sup>): NO<sub>3</sub>-N (as KNO<sub>3</sub>)—600; PO<sub>3</sub><sup>3-</sup> (as Na<sub>2</sub>HPO<sub>4</sub> × 12H<sub>2</sub>O and KH<sub>2</sub>PO<sub>4</sub>)—700; Mg<sup>2+</sup> (as MgSO<sub>4</sub>)—2.4, methanol 1900. Operation of denitrifying unit and the isolation and identification methods were described in our previous papers (Grabińska-Łoniewska et. al., 1985; Sláviková and Grabińska-Łoniewska, 1988 (1989)).

All strains are deposited in the Czechoslovak Collection of Yeasts (CCY), Institute of Chemistry SAS, Bratislava.

## RESULTS AND DISCUSSION

8 strains of yeasts and yeast-like microorganisms have been isolated and identified in a anaerobic denitrifying sludge fed with a mineral medium containing methanol as a carbon source. They were classified as: *Candida boidinii*, *Candida maltosa*, *Rhodotorula rubra* and *Trichosporon cutaneum* according to principles suggested by Kočková-Kratochvilová (1984), Kočková-Kratochvilová and Sláviková (1985), Komagata et. al. (1964); Lodder (1970) and Meyer et. al. (1975).

Description of the cell and culture morphology and some physiological characters of those strains is given below. Fermentation and assimilation of different sugars and assimilation of some compounds is summarized in Table 1.

*Candida boidinii* Ramirez — strains M3. D2, M4. D3 and M18, D3 (CCY 29-37-8; 29-37-6 and 29-37-7, respectively). \*

The cells are long-ovoid to cylindrical (Figs. 1 a,b,c). During the growth in liquid medium a sediment and a pellicle are formed. The giant colonies are cream-coloured, dull, soft and wrinkled, (Fig. 2a). Radial growth rate in 100 hrs at 20 °C is: strain M3.D2-4.39 mm; strain M4.D3-4.60 mm; strain M18.D3-5.04 mm. Pseudomycelium is abundantly formed. Sporulation is not observed. All strains do not produce of urease. They grow weak in vitamin-free medium. At the temperature 28 °C they grow well, the growth of the strains M3.D2 and M18.D3 at 37 °C is poor while strain M4.D3 does not grow at all.

*Candida maltosa* Komagata, Nakase et Katsuya — strains M6.D7 and M18.D7 (CCY 29-88-3 and 29-88-4, respectively).

The shape of the cell is round to short oval (Fig. 1 d,e). They usually occur single or in pairs. In a culture in liquid medium a ring and a sediment

\* ) Number of strains preserved in Czechoslovak Collection of Yeasts (CCY)

Table 1  
Physiological characteristics of the isolated strains

Character of strains	<i>C. boidinii</i> (M3. D2 M4. D3 M18. D3)	<i>C. maltosa</i> (M6. D7 M18. D7)	<i>Rh. rubra</i> (M4. D4 M11. D4)	<i>T. cutaneum</i> (M5. D5)
Fermentation:				
Mal	—	+	—	—
		(weak)		
Sac	—	+	—	—
Lac	—	—	—	—
Glc	+	+	—	—
Assimilation:				
Mal	—	+	+	+
Sac	—	+	+	+
Lac	—	—	—	+
Raf	—	—	+	+
MLz	—	+	+	—
D-Xyl	+	+	+	+
L-Ara	—	—	+	+
Inl	—	—	—	—
Aml	—	—	—	—
Cel	—	+	—	+
Tre	—	+	+	+
KNO <sub>3</sub>	+	—	—	—
Ethanol	+	/	/	/
Methanol	+	/	/	/

Abbreviations: Glc — glucose; Mal — maltose; Sac — sucrose; Lac — lactose; Raf — raffinose; MLz — melezitose; D-Xyl — D-xylose; L-Ara — L-arabinose; Inl — inulin; Aml — amylose; Cel — cellobiose; Tre — trehalose; — test negative; + test positive; / not investigated

are formed. Colonies are cream-coloured, flat, smooth and soft (Fig 2b), radial growth rate in 100 hrs at 20°C: strain M6.D7-3,95 mm, strain M18.D7-3,51 mm. Formation of pseudomycelium is comparatively reduced. Sporulation is not observed. They do not hydrolyse the urea and don't grow in vitamin-free medium. The growth at 28 and 37°C is good, but they do not grow at 42°C.

*Rhodotorula rubra* (Demme) Lodder — strains M4.D4 and M11.D4 (CCY 20-7-15 and 20-7-14, respectively).

The cells are short-ovoidal to elongate. They occur single or in pairs (Fig. 1 f, g). In a liquid medium a pink-coloured ring and little sediment are formed. They form pink-coloured giant colonies with a glistening and smooth surface (Fig. 2c). The radial growth rate in 100 hrs at 20°C is: strain M4.D4-4.82 mm, strain M11.D4-4.60 mm. The pseudomycelium and spo-

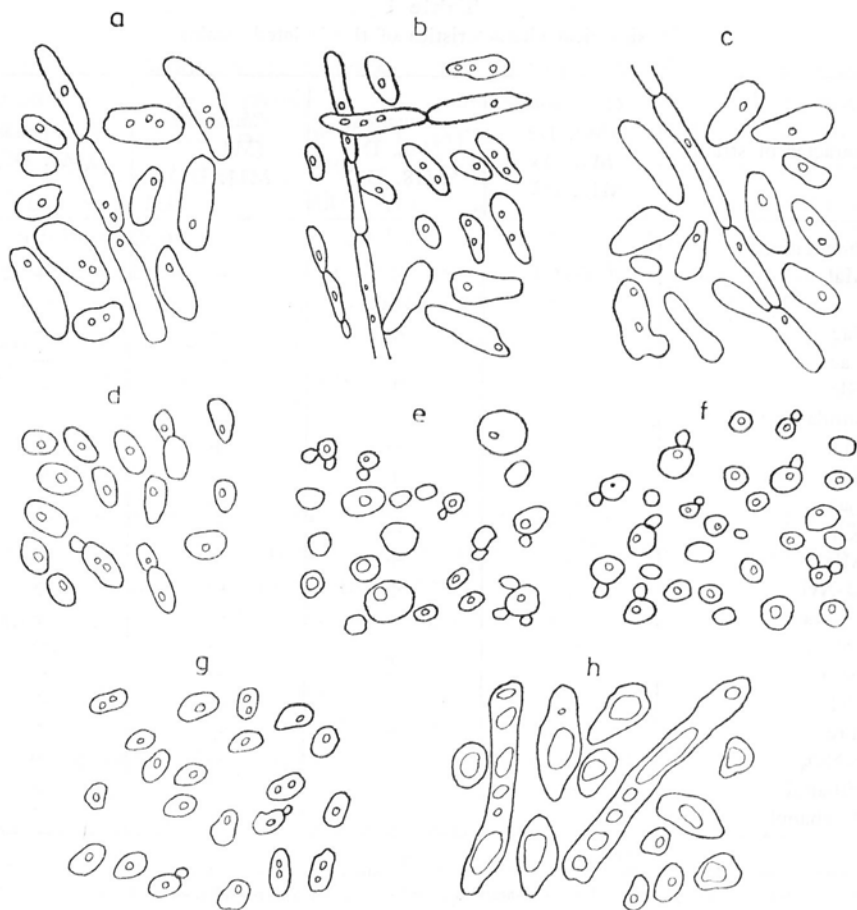


Fig. 1. Cell morphology (magnification 900x)

a c — *Candida boidinii* (strains: M3.D2; M4.D3; M18.D3, respectively); d-e — *C. maltosa* (strains M6.D7 and M18.D7, respectively); f-g — *Rhodotorula rubra* (strains M4.D4 and M11.D4, respectively); h — *Trichosporon cutaneum* (strain M5.D5)

ulation are not observed. They are able to hydrolyse urea, but don't grow in vitamin-free medium. They grow good at 28 and 37°C; the growth at 5°C is weak.

*Trichosporon cutaneum* (de Berum, Gougerot et Vaucher) Ota — strain M5.D5 (CCY 30-5-14).

The shape of cells is ovoid or ellipsoidal, sometimes shoesole like (Fig. 1h). During the growth in liquid medium a pellicle is formed, growth is dull, cream-coloured, wrinkled, thick, sometimes tending to sink in medium. The surface of giant colonies is wrinkled, rugose, cream-coloured, hairy, with tough texture, radially folded border (Fig. 2d). The radial growth rate is 20,83 mm

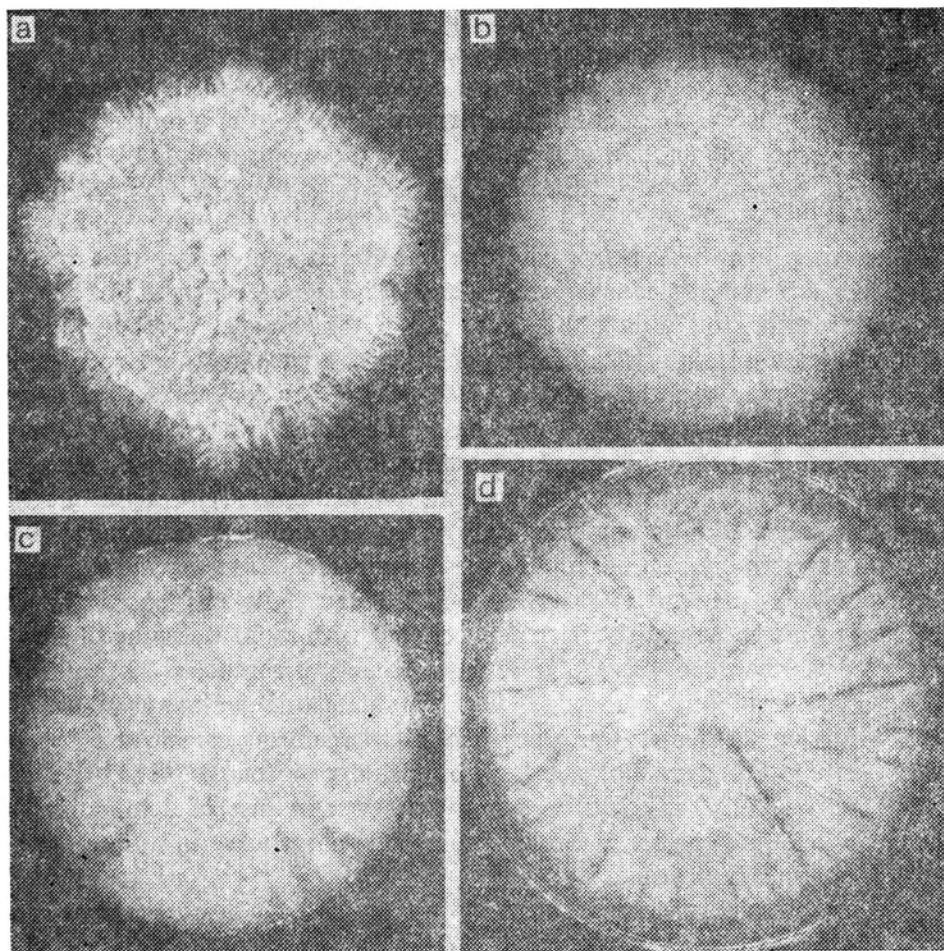


Fig. 2. Giant colonies

a — *Candida boidinii* (strain M4.D3); b — *C. maltosa* (strain M18.D7); c — *Rhodotorula rubra* (strain M4.D4);  
d — *Trichosporon cutaneum* (strain M5.D5)

in 100 hrs at 20°C. This organism forms pseudomycelium and true mycelium. Sporulation is not observed. Urease is produced, they show a weak growth in vitamin-free medium. The strain grows well at 28°C, but at 37°C does not.

Among described above species *Candida boidinii* can be regarded as the most specific fungal organism for the denitrifying unit biocenosis fed with methanol. Representatives of this species existed in the sludge during the whole eight months period of studies carried out at reactor load in range 220-1800 mgNO<sub>3</sub>-N l<sup>-1</sup> day<sup>-1</sup>. It was stated that those organisms consti-

tuted 75-94% of total amount of fungi found in the sludge (Grabińska-Łoniewska, 1990). Basing on the physiological characteristics of these species (Table 1) it could be said that the growth substrate for them were both methanol and nitrates present in a medium used as a feed in a course of denitrification process. The ability of utilization of those compounds by *Candida boidinii* has been described earlier by many authors (e.g. Sahm, Wagner 1972).

The other identified species could be considered as a concomitant microorganisms. *Trichosporon cutaneum* and *Rhodotorula rubra* developed in a sludge at a load from 220 to 1330 mg  $\text{NO}_3\text{-N l}^{-1} \text{ day}^{-1}$ . They made, respectively 2-15 and 2-5% of total amount of fungal cells. Appearance of *Candida maltosa* was observed at a load 330-1800 mg  $\text{NO}_3\text{-N l}^{-1} \text{ day}^{-1}$ . Representatives of this species composed 2-13% of total amount of fungi. They had not ability to assimilate nitrates and they need vitamins for growth. So it could suggest that the growth substrate for them was not the medium fed in reactor but the compounds released from autolyzed denitrifying bacterial cells. This suggestion was confirmed by the fact of rapid increase of representatives of *Candida maltosa* in sludge at the load 1800 mg  $\text{NO}_3\text{-N l}^{-1} \text{ day}^{-1}$ . At this load a violent decrease of nitrate removal efficiency coupled with rapid decaying of denitrifying bacteria were observed. Then those organisms composed 25% of total fungal organisms found in a sludge (Grabińska-Łoniewska, 1989).

Considering that yeasts and yeast-like microorganisms constituted merely 0,003-0,010% of total amount of organisms of sludge biocenosis (Grabińska-Łoniewska, 1989) it could be stated that even in case of domination of nitrate assimilating species, the role of those microorganisms in nitrate removal process isn't significant.

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**Badania taksonomiczne nad drożdżami i grzybami  
drożdżopodobnymi wyizolowanymi z biocenozy urządzenia  
do denitryfikacji ścieków**

Streszczenie

Przebadano osiem szczepów drożdży i grzybów drożdżopodobnych wyizolowanych z biocenozy zasiedlającej urządzenie do denitryfikacji ścieków zawierających metanol jako źródło węgla.