Torulopsis apis var. *galacta* var. nov.

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A description has been given of a new variety of yeast associated with the ants of the group *Formica rufa* L. *Torulopsis apis* var. *galacta* differs from *T. apis* by its spheroidal cell shape and its inability (or very weak ability) to assimilate raffinose and to grow in vitamin-free medium. In addition, it was found that the type strain *T. apis* produces primitive pseudomycelium.

In the report on the yeast species associated with ants of the group *Formica rufa* (1), reference was made to an asporogenous yeast which was discovered on cocoons. During the course of subsequent microbiological study on anthills, this yeast was isolated repeatedly, although it was never again found in large numbers. Other than on cocoons, it was found on the material of the inner cone of anthills and in the intestine of females.

This yeast is similar in its characteristics to *Torulopsis apis* Lavie ex van Uden et Vidal-Leiria (4) isolated from bees, but it cannot be identified with this species, mainly because of morphological differences. The following description of the new variety, *T. apis* var. *galacta*, is based on the study of 14 strains.

MATERIALS AND METHODS

Methods for isolation of yeasts from anthills have been given previously (1).

The description of the new variety is based on the standard methods employed in yeast taxonomy (5). For comparison, the type strain of *T. apis* BKM Y-1538 (CBS 2674) was used.

The extraction of deoxyribonucleic acid (DNA) and chemical determination of base composition were carried out by Vanushin's modification of the method of Schmidt and Thanhauser (7).

DESCRIPTION

Torulopsis apis Lavie ex van Uden et Vidal-Leiria var. *galacta* Golubev et Bab'eva nov. var.

Haec varietas a specie differt: Cellulae rotundae. In malto agaro cultura (post unum mensem, 20°C) alba. Pseudomycelium non format. Raffinosum non assimilantur (interdum exigue). Vitaminae additae necessariae sunt.

Typus culture: IBPhM Y-708 in collectione Instituti Biochemiae et Physiologie Microorganismorum Acad. Sci. URSS (Pushchino) conservatur, isolata ex pupulis *Formica rufa* L., 1970.

Growth in liquid media (yeast morphology

medium [Difco], glucose-yeast extract-peptone water, or malt extract): After 3 days at 25°C the cells are spheroidal, 2 to 4.5 μm , single, in pairs, small clusters. The budding is multilateral; several buds may be present on the mother cell at the same time. No capsule demonstrable.

After 1 month at 20°C a sediment and a thin ring are present.

Growth on solid media (yeast morphology agar [Difco], glucose-yeast extract-peptone agar, or malt agar): After 3 days at 25°C the cells are of the same shape as in liquid media, 1.6 to 4 (rarely up to 6 μm) (Fig. 1).

After 1 month at 20°C the streak cultures are pasty, white, mat, smooth, slightly wrinkled on the periphery. The margin is entire.

Growth on malt gelatin: After 1 month at 17°C the colony is white, dull, pasty, smooth, slightly convex. The margin is entire. Gelatin is not liquefied.

Slide cultures on yeast morphology agar (Difco), cornmeal agar, and potato-glucose agar: Neither pseudomycelium nor true mycelium is produced.

Ascospore, ballistospore and teliospore formation: Not observed in single and mixed cultures.

Fermentation: Absent.

Assimilation of carbon compounds: Glucose, +; galactose, + weak; L-sorbose, - or + very weak; sucrose, + weak; maltose, -; cellobiose, -; trehalose, -; lactose, -; melibiose, -; raffinose, - or + very weak; melezitose, -; inulin, -; soluble starch, -; D-xylose, -; L-arabinose, -; D-arabinose, -; D-ribose, -; L-rhamnose, -; ethanol, -; glycerol, + or weak; erythritol, -; ribitol, -; galactitol, -; D-mannitol, +; D-glucitol, +; α -methyl-D-glucoside, -; salicin, -; DL-lactic acid, -; succinic acid, + weak; citric acid, -; i-inositol, -; glucuronic acid, -.

Splitting of arbutin: Negative.

Assimilation of potassium nitrate: Negative.
Urease activity: Negative.

Formation of starch-like material: Absent.

Growth on vitamin-free medium: Absent.

Growth at 37°C: Absent.

Acid formation on chalk agar: Absent.

Growth on 50% (wt/wt) glucose-yeast extract agar: Absent. Slow growth on 35% glucose.

Maximum concentration of sodium chloride allowing slow and weak growth: 5 to 6%.

Guanine-plus-cytosine content in nuclear DNA: 46.0 ± 0.5 mol%.

Etymology: *galacta* from the L. adj., milk-white; referring to the color of the streak culture.

The type culture is IBPhM Y-708. It has been deposited in the collection of microorganisms, Institute of Biochemistry and Physiological Microorganisms, U.S.S.R. Academy of Sciences, Pushchino, Moscow region, U.S.S.R. This culture was isolated from cocoons of *Formica rufa* L., 1970.

DISCUSSION

The new yeast belongs to the group of organisms of the genus *Torulopsis* Berlese unable to utilize nitrate and to ferment any sugars. This group has been enlarged in recent years

mainly by descriptions of new species associated with various insects (2, 6).

The absence of urease activity, guanine-plus-cytosine (G + C) content, and the cell wall structure (V. V. Dmitriev, personal communication) strongly suggest that *T. apis* var. *galacta* is an ascomycetous yeast. The G + C value for the new variety is close to that for *T. apis* — 48.8 mol% (3).

T. apis var. *galacta* and *T. apis* show great similarity in their carbon assimilation tests. However, it would appear feasible to distinguish them on the basis of raffinose utilization and the ability to grow in vitamin-free medium. Both *T. apis* var. *galacta* and *T. apis* BKM Y-1538 utilize succinic acid weakly.

In contrast to their close physiological similarity, *T. apis* and *T. apis* var. *galacta* clearly differ in morphology. The cells of the former are oval (Fig. 2), whereas the cells of the latter are spheroidal (Fig. 1). The difference in the shape of cells is sharper on glycerol and in aging cultures. Moreover, the type strain of *T. apis* was found to form rudimentary pseudomycelium (Fig. 3), unlike that of *T. apis* var. *galacta*. In addition, the streak cultures of the

FIG. 1. *Torulopsis apis* var. *galacta* IBPhM Y-708 (morphology agar, 20°C, 3 days, phase contrast, magnification $\times 900$).

FIG. 2. *Torulopsis apis* BKM Y-1538 (morphology agar, 20°C, 3 days, phase contrast, magnification $\times 900$).

FIG. 3. Pseudomycelium of *Torulopsis apis* BKM Y-1538 (slide culture on morphology agar, 20°C, 10 days, phase contrast, magnification $\times 900$, stained with cotton blue).

latter remain white after 1 month, but the culture of *T. apis* becomes light brown and later darkens even more. The above-mentioned differences and the great similarity of these yeasts allow us to consider our isolates to be a new variety of *T. apis*. Production of rudimentary pseudomycelium is obviously of little or no value for separation and, therefore, we are not proposing to transfer *T. apis* to the genus *Candida* Berkhout or to consider the new yeast a separate species.

REPRINT REQUESTS

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