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Growth of *Candida famata* and *Trichosporon cutaneum* on uric acid as the sole source of carbon and energy, a hitherto unknown property of yeasts

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Yeast strains capable of utilizing uric acid as the sole source of carbon and energy were isolated from soil by the enrichment culture method. The strains were identified as *Candida famata* (Harrison) Meyer et Yarrow and *Trichosporon cutaneum* (De Beurm., Gougerot et Vaucher) Ota. On the subcellular level growth of yeasts on uric acid was accompanied with the development of a number of large microbodies in the cells.

INTRODUCTION

Utilization of purines as a source of nitrogen is a widespread property of yeasts (LaRue and Spencer, 1968). In yeasts and fungi and in several aerobic bacteria purine bases are metabolized via xanthine, uric acid, allantoin, allantoate and ureidoglycolate to glyoxylate and urea (see review by Vogels and Van der Drift, 1976). Urea is hydrolysed by urease to ammonia and bicarbonate or – in urease-negative yeasts – carboxylated to allophanate which subsequently is hydrolysed to ammonia and bicarbonate (Roon and Levenberg, 1970; Whitney and Cooper, 1972).

Uric acid is an energy-poor substrate. If its metabolism proceeds via allantoin, glyoxylate liberated from the latter provides the only energy-yielding carbon compound. The glyoxylate molecule has the same redox level as formaldehyde, only two oxidation steps removed from carbon dioxide. In spite of the low energy content, uric acid and allantoin support growth of many bacteria and fungi when administered as the sole source of carbon and energy (Vogels and Van der Drift, 1976). Growth, under these conditions, of yeasts has never been reported. In the present paper we report the isolation from soil of yeasts which are able to grow with uric acid as the sole source of carbon and energy.

Materials and Methods

Enrichment of uric acid-assimilating yeasts

The basal growth medium contained per litre demineralized water: 10 g uric acid, xanthine of adenine, 1 g potassium dihydrogen phosphate, 0.1 g magnesium sulphate, trace elements and vitamins at a concentration 20 % of that reported earlier (Middelhoven, 1969), pH 5.0. Bacterial growth was suppressed by the addition of 200 mg streptomycin sulphate, 200 mg bacitracin, 10 mg chloramphenicol, 10 mg tetracyclin hydrochloride, 200 mg penicillin G. The medium (80 ml in a 300-ml Erlenmeyer flask) was inoculated with a spoonful of soil, taken from grassland, a tropical greenhouse or a chicken-run. Incubation took place at 30 °C in a rotary shaker. Occasionally the pH was adjusted by the addition of hydrochloric acid. When large numbers of bacteria were observed microscopically the pH was temporarily decreased to 3.0. Degradation of purines was deduced from the disappearance of the white crystals of the substrate, which usually occurred within a week. One ml of such a culture was then transferred to 80 ml fresh medium of the same composition which was incubated as described above. Crystals usually disappeared after 2 to 3 days. The cultures were streaked pure on malt agar or on basal growth medium agar (20 g agar$\cdot l^{-1}$) with 10 g uric acid$\cdot l^{-1}$ or a physiologically related substance such as glycine, allantoin or ethylamine and ethanol as the sources of carbon, nitrogen and energy. Yeast cultures were maintained on malt agar slants.

The yeast isolates were identified according to Lodder (1970). Some of their properties were compared with those of strains from the culture collection of the Centraalbureau voor Schimmelcultures in Delft, viz. *Debaryomyces hansenii* CBS G815, CBS G817 and CBS G856 and *Trichosporon cutaneum* CBS5790 and CBS2466 (type strain).

Electron microscopy

Yeast cells were harvested, washed with distilled water and subsequently fixed in potassium permanganate (15 g$\cdot l^{-1}$) for 20 min at room temperature. After dehydration in a graded ethanol series, the cells were embedded in Epon 812.

Ultrathin sections were cut with a diamond knife and examined in a Philips EM 300 electron microscope.

RESULTS

Inoculation of uric acid or xanthine basal growth medium with soil as described above usually resulted in growth of microorganisms within a week of incubation. Filamentous fungi predominated. In spite of the presence of five antibiotics bacterial growth was not completely suppressed. Microscopically, yeasts were usually difficult to detect in the first crude enrichment cultures but were present in the subsequent cultures in enrichment medium. When the latter culture was streaked on malt agar, yeasts of several colony types developed. However, many of these strains were unable to grow in pure culture on uric acid as the sole source of carbon and energy. Most probably these organisms had grown in the enrichment culture on substances excreted by uric acid-assimilating filamentous fungi. Streaking of the enrichment culture on selective agar media, e.g. basal growth medium agar with uric acid as the sole carbon source, was a more direct way to isolate uric acid-assimilating yeast strains. Attempts to isolate strains capable of growth on xanthine as the sole carbon source have yet failed. From enrichment cultures on adenine, however, adenine-assimilating yeast strains were isolated which belong to a newly defined *Trichosporon* species and will be described elsewhere (Middelhoven et al., to be published).

The uric acid-assimilating yeast strains were identified as *Candida famata* (Harrison) Meyer et Yarrow and *Trichosporon cutaneum* (De Beurm., Gougerot et Vaucher) Ota according to generally accepted taxonomic criteria (Lodder, 1970). The former species is the imperfect state of *Debaryomyces hansenii* (Zopf.) Lodder et Kreger-van Rij which is an ascomycete. *T. cutaneum* is an imperfect yeast. It is concluded to be basidiomycetous because of the ultrastructure of its cell wall.

C. famata strain TOX-2 was isolated from an enrichment culture on xanthine inoculated with soil from a tropical greenhouse. The strain did not grow on xanthine as the sole carbon source but did grow on uric acid faster than any of the *T. cutaneum* isolates. In uric acid basal growth medium a 1% inoculum grown in the same medium needed 18–24 h to attain the stationary growth phase; thus the growth rate was roughly estimated to be about 0.19–0.26 h^{-1}. Growth on uric acid is not a common property among strains of *D. hansenii*. Strains CBS G815, CBS G817 and CBS G856 belonging to this yeast species were unable to grow in uric acid basal growth medium.

Strain TOX-2 showed spherical to ovoid cells (3.5–5.5 µm) when grown on malt agar slants (Fig. 1a). In liquid uric acid basal growth medium cells were smaller (2.5–3.5 µm, Fig. 1b). In old cultures on malt agar slants some slender cells and buds were observed (Fig. 1c). Ascospores were not observed. Strain TOX-2 differed from most of the other strains of *C. famata* and *D. hansenii*

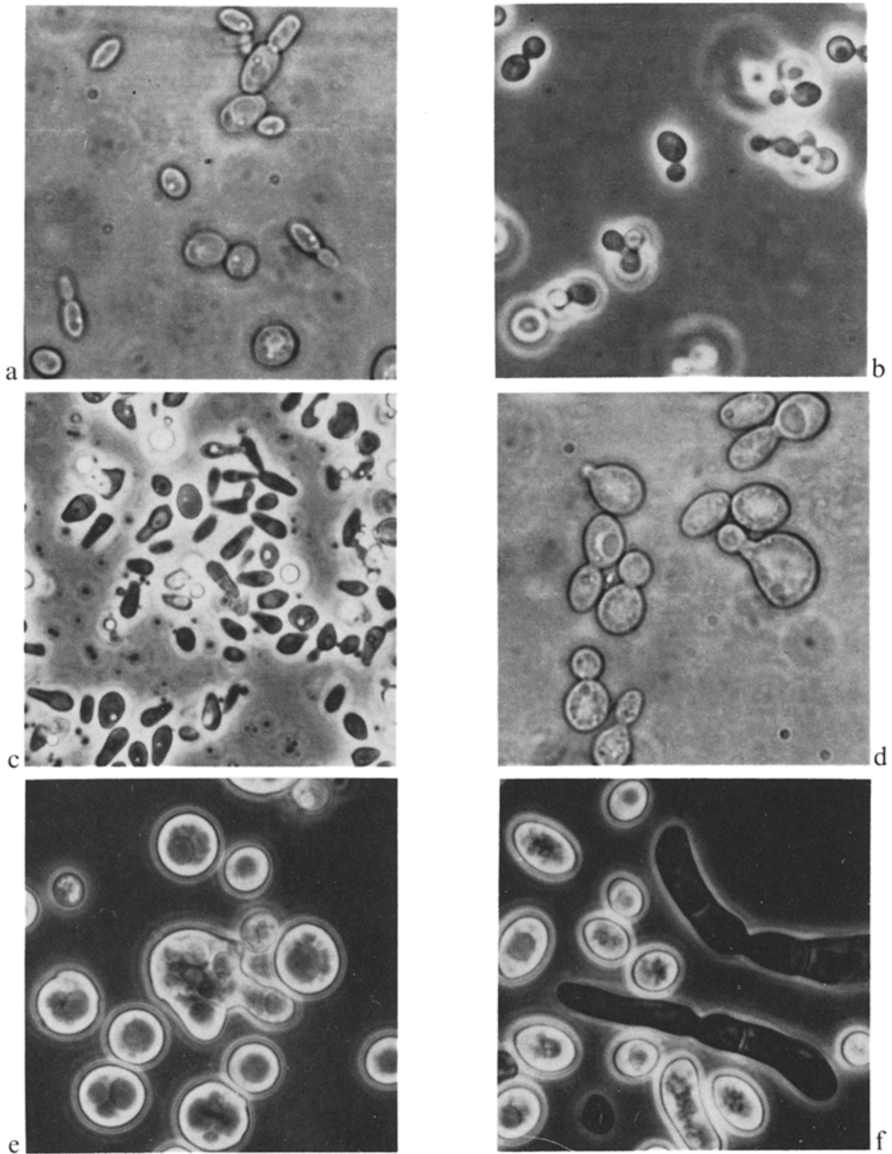

Fig. 1. Micrographs of uric acid-assimilating yeasts. Magnification 1250 ×.
(a) *Candida famata* strain TOX-2, malt agar slant, 18 h; (b) id., liquid uric acid basal growth medium, 24 h; (c) id., malt agar slant, 4 weeks;
(d) *Trichosporon cutaneum* strain KOU-26, malt agar slant, 18 h; (e) id., malt agar slant, 4 weeks; (f) id., freshly isolated, malt agar slant, 4 weeks.

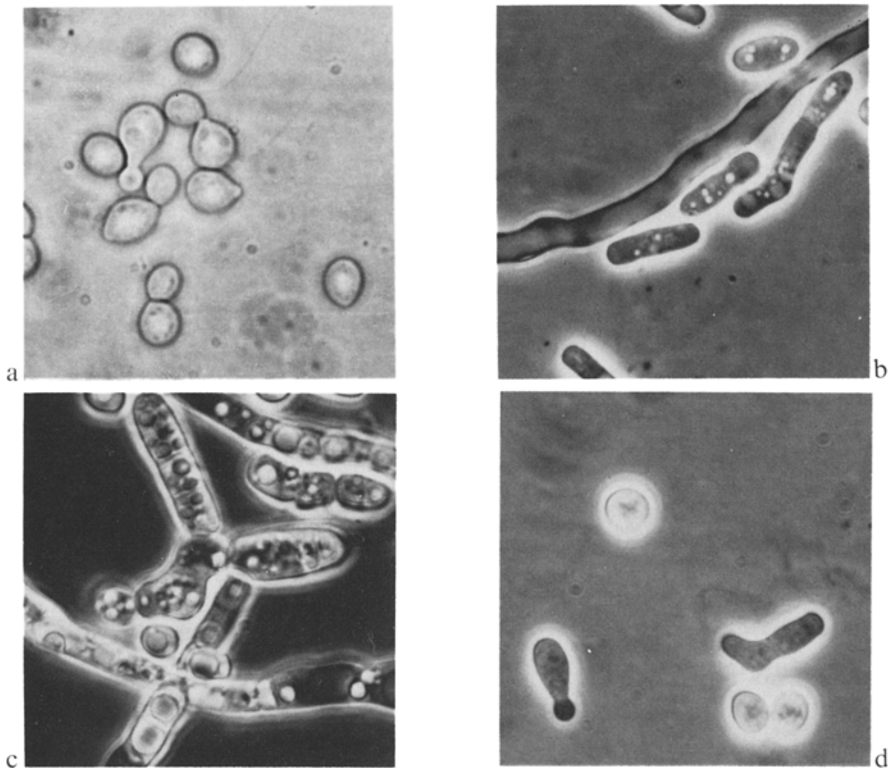

Fig. 2. Micrographs of uric acid-assimilating yeasts. Magnification 1250 ×.
(a) *Trichosporon cutaneum* strain KOU-26, liquid uric acid basal growth medium, 48 h;
(b) *T. cutaneum* strain TOU-17, malt agar slant, 24 h; (c) id., malt agar slant, 1 week; (d) id., liquid uric acid basal growth medium 48 h.

in producing more pseudomycelium on corn meal agar (not shown) and in failing to assimilate L-arabinose. Cellobiose assimilation was very slow and arbutin hydrolysis was negative.

T. cutaneum strains KOU-26 and TOU-17 were isolated from enrichment cultures in uric acid basal growth medium inoculated with soil taken from a chicken-run and with garden soil, respectively. In liquid uric acid basal growth medium a 1% inoculum grown in the same medium needed 36–48 h to attain the stationary growth phase; thus the specific growth rate was roughly estimated to be about 0.09–0.12 h^{-1}.

Strain KOU-26 grown on malt agar slants showed spherical or ovoid cells (5.5–8 μm) budding on a broad base (Fig. 1d). After 4 weeks of incubation of the malt agar slants, large spherical cells predominated (Fig. 1e). However, when freshly isolated, strain KOU-26 produced pseudomycelium on malt agar slants (Fig. 1f). Cells grown in liquid uric acid basal growth medium were ovoid and

somewhat smaller (4.5–6.5 μm) than cells grown on malt agar slants.

Strain TOU-17 differed from strain KOU-26 in producing more pseudomycelium and mycelium, especially when grown on malt agar slants (Fig. 2b and c). During growth in liquid uric acid basal growth medium strain TOU-17 produced both spherical and elongated cells (Fig. 2d) in contrast to strain KOU-26 that showed only ovoid cells (Fig. 2a).

Growth on uric acid as the sole source of carbon and energy is a property shown by more strains of *T. cutaneum.* Strain VIII-1, isolated many years ago from activated sludge grown on arginine synthetic medium, also grew on uric acid though slower than strains KOU-26 and TOU-17. Strain CBS5790 grew on uric acid very well, but strain CBS2466 (type strain of the species) failed to do so.

Both strains showed a number of common properties. They grew rapidly on glucose, maltose, sucrose, galactose and lactose, but not on starch or on 50% glucose yeast extract agar, could not assimilate potassium nitrate and did not show fermentation of glucose. Furthermore, compounds metabolically related to glyoxylate, viz. glycolate or ethylene glycol were not assimilated as the sole source of carbon and energy. Both strains differed with respect to some other properties. Strain TOU-17 grew very well on citrate and strain KOU-26 on lactate. Strain TOU-17 did not assimilate erythritol or raffinose but was more thermotolerant and osmotolerant than strain KOU-26. It grew at 37 °C but not at 40 °C and tolerated 10% sodium chloride in malt agar. Strain TOU-17 utilized caseine, gelatin and ethylamine as the sole source of carbon and energy.

Electron microscopical observations of thin sections of potassium permanganate-fixed cells revealed that during growth of yeasts on uric acid a number of large microbodies had developed in the cells (Figs 3a and b), which were absent in cells grown on glucose ammonia medium (Fig. 3c). The microbodies were irregular in shape and frequently appeared in clusters (Figs 3a and b).

Discussion

Uric acid is the main nitrogenous excretory product of several animals, e.g. of birds and reptiles. Hence it is present in nature, especially in terrestrial ecosystems. Degradation of uric acid in soil is effected by several aerobic and anaerobic bacteria and by filamentous fungi (Vogels and Van der Drift, 1976) which are able to utilize the compound as the sole source of carbon and energy. Many other organisms, yeasts included (LaRue and Spencer, 1968) can utilize uric acid as the nitrogen source and may take a part in the degradation of the compound in soil if a source of carbon and energy is available. Yeasts that can utilize uric acid as the sole source of carbon and energy were yet unknown. In the present paper we report the isolation of such yeast strains from soil. They were shown to belong to two species, viz. *C. famata,* which is the imperfect state of *D. hansenii,* and *T. cutaneum,* which is a basidiomycetous imperfect yeast.

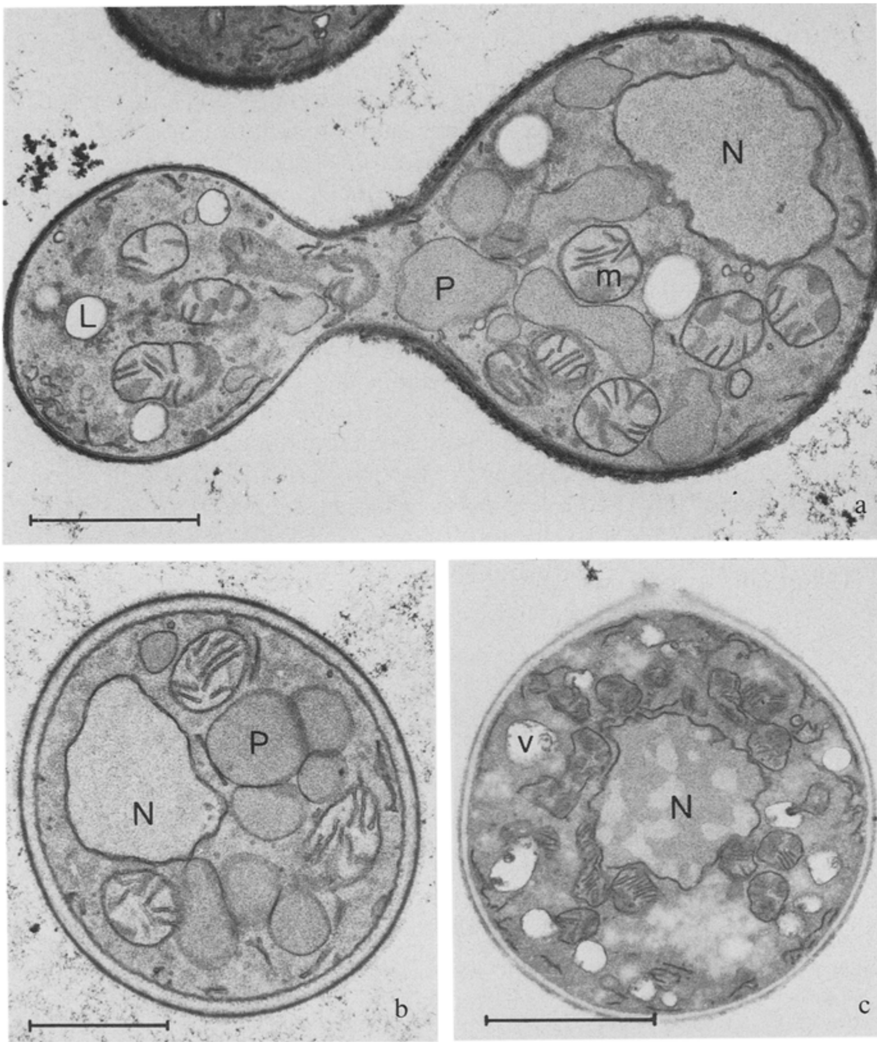

Fig. 3. Electron micrographs of thin sections or permanganate-fixed cells of (a) *Trichosporon cutaneum* strain KOU-26 and (b) *Candida famata* strain TOX-2, grown in liquid uric acid basal growth medium. The cells contain a number of irregularly shaped microbodies (P) which are absent in (c) cells of *T. cutaneum* strain KOU-26, grown in glucose ammonia medium.
L = lipid droplet, m = mitochondrion, N = nucleus, V = vacuole; the bars represent 1.0 μm.

It is at present difficult to assess to which extent yeasts take part in the metabolism of uric acid in nature. In the soil numbers of yeast cells which are able to utilize uric acid as the sole source of carbon and energy appear to be small.

Enrichment cultures are usually dominated by filamentous fungi or bacteria. So the role of *C. famata* and *T. cutaneum* in the dissimilation of uric acid in soil is probably limited.

Growth on uric acid as the sole source of carbon, energy and nitrogen is characteristically associated with the development of a number of large microbodies in the cell. Their absence in glucose-grown cells suggests that these organelles are involved in carbon and/or nitrogen metabolism. Since urate oxidase, the key enzyme in aerobic uric acid metabolism, is a typical peroxisomal enzyme, which is for instance present in yeast peroxisomes during growth of cells on uric acid as the nitrogen source (Veenhuis et al., 1980), these organelles most probably are peroxisomes. Yeast peroxisomes have been shown to be the site of several enzymes involved in the catabolism of carbon and nitrogen compounds, e.g. alcohol oxidase, D-amino acid oxidase and amine oxidase (see review by Veenhuis et al., 1983).

The strains described have been deposited in the culture collection of the Centraalbureau voor Schimmelcultures (Yeast Division) in Delft, *Candida famata* strain TOX-2 as CBS 8109, *Trichosporon cutaneum* strain KOU-26 as CBS 8110 and strain TOU-17 as CBS 8111.

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