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Trichosporon adeninovorans sp. nov., a yeast species utilizing adenine, xanthine, uric acid, putrescine and primary *n*-alkylamines as the sole source of carbon, nitrogen and energy

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A new yeast species, *Trichosporon adeninovorans*, was isolated from soil by the enrichment culture method. Apart from adenine, the strain utilized uric acid, guanine, xanthine, hypoxanthine, 6,8-dihydroxypurine, putrescine, propylamine, butylamine, pentylamine, hexylamine and octylamine as sole source of carbon, nitrogen and energy.

The structure of the cell wall of *Tr. adeninovorans* was ascomycetous. On the subcellular level growth on adenine or uric acid was accompanied with the development of microbodies in the cell. These cell organelles probably were the site of urate oxidase, an enzyme that, after growth on purine substrates, together with allantoinase was present at high activities. Low activities of adenine amidohydrolase and xanthine dehydrogenase were also demonstrated.

INTRODUCTION

The isolation from soil of two yeast species, viz. *Candida famata* and *Trichosporon cutaneum*, utilizing uric acid as the sole source of carbon, nitrogen and energy has recently been described (Middelhoven et al., 1983). Enrichment cultures on adenine yielded yeast strains, all belonging to one type, which were able to grow on adenine or uric acid as the sole carbon source (Middelhoven

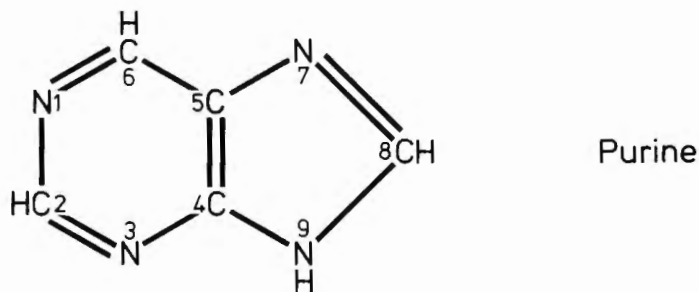


Fig. 1. Structure of substituted purine compounds. Adenine = 6-aminopurine; hypoxanthine = 6-hydroxypurine; guanine = 2-amino-6-hydroxypurine; isoguanine = 6-amino-2-hydroxypurine; xanthine = 2,6-dihydroxypurine; uric acid = 2,6,8-trihydroxypurine.

et al., 1983). As the properties of the adenine-utilizing yeast strains differed from those of all yeast species hitherto known, a novel yeast species is defined in the present paper. Moreover, some information on the pathway of adenine catabolism is presented.

MATERIALS AND METHODS

Yeast strains

Trichosporon adeninovorans TOA-3 was isolated from an enrichment culture with adenine as the sole source of carbon, nitrogen and energy which had been inoculated with soil taken from a chicken-run (Middelhoven et al., 1983). The strain was maintained on malt agar slants. Only strain TOA-3 was studied in detail since the other adenine-utilizing yeast strains isolated from the enrichment culture were identical with respect to cell and colony morphology and a number of other properties.

For comparison, some parallel experiments were run with the type strain of a possibly related species, *Trichosporon terrestris* CBS 6697 (Van der Walt and Johannsen, 1975). The methods used in the standard description of strain TOA-3 were in accordance with generally accepted taxonomic criteria (Van der Walt, 1970), and the assimilation of hydrocarbons was studied according to Bos and De Bruyn (1973).

Cultivation

The basal growth medium that had been used in the enrichment cultures was used throughout the investigation, but the concentration of KH_2PO_4 was raised to $20 \text{ g} \cdot \text{l}^{-1}$ to improve the buffering capacity. The culture was usually adjusted to pH 5.0, but to pH 6.5 or 7.0 when xanthine was the carbon source. Growth usually took place in 100 ml medium in a 300-ml Erlenmeyer flask, incubated on a rotatory shaker at 37°C . For some biochemical experiments the cells were grown in a Kluver flask at 37°C . The utilization of the purine substrate was

deduced from the decrease in UV absorbance of the growth medium.

Chemicals

Isoguanine, 2,8-dihydroxyadenine, xanthine and allopurinol were purchased from Sigma; 6,8-dihydroxypurine was prepared by Dr N. J. Kos, Department of Organic Chemistry, Agricultural University, Wageningen; 8-hydroxyadenine was prepared by Mr H. Jongejan from 4, 5, 6-triaminopyrimidine and urea according to Robins (1958); 8-phenylhypoxanthine was a gift of Prof. Dr H. C. van der Plas. The structures of the substituted purine compounds used are shown in Fig. 1.

Enzyme assays

Cells (100 to 200 mg dry biomass) were suspended in 2.5 ml 50 mM potassium phosphate buffer, pH 7.0, and stored at -20°C . Sonic disruption was achieved by treatment with an M.S.E. Ultrasonic Power Supply for 3 to 4 min at 0°C . The crude extract was centrifuged to remove cell debris. The supernatant was used as a cell-free extract. Protein was determined by a modified Lowry method (DeMoss and Bard, 1957). Catalase (H_2O_2 : H_2O_2 oxidoreductase, EC 1.11.1.6) was determined spectrophotometrically at 240 nm by following the enzyme-catalysed decomposition of 4 mM hydrogen peroxide in 0.1 M potassium phosphate buffer, pH 7.0. Urate oxidase (urate: O_2 oxidoreductase, EC 1.7.3.3) was determined spectrophotometrically by following the decrease of the absorbance at 293 nm in an assay mixture containing 0.1 mM uric acid and 0.1 M Tris-HCl buffer, pH 9.0. Allantoinase (allantoin amidohydrolase, EC 3.5.2.5) was determined according to Middelhoven (1977). The dehydrogenation of various purine substrates by cell-free extracts was studied with either O_2 or NAD^+ as the electron acceptor. In the oxidase assays cell-free extract was added to 0.1 mM purine substrate dissolved in 0.1 M Tris-HCl buffer, pH 8.0. The decrease in UV absorbance was followed spectrophotometrically: hypoxanthine at 250 nm, xanthine at 269 nm, 6,8-dihydroxypurine at 260 nm and 8-hydroxyadenine at 278 nm.

The dehydrogenase assays were similar to those of the oxidase reactions, 0.1 mM NAD^+ being added to the latter. NADH production was recorded at 340 nm. Corrections were made for endogenous NADH production, which usually was negligible, or for NADH oxidase.

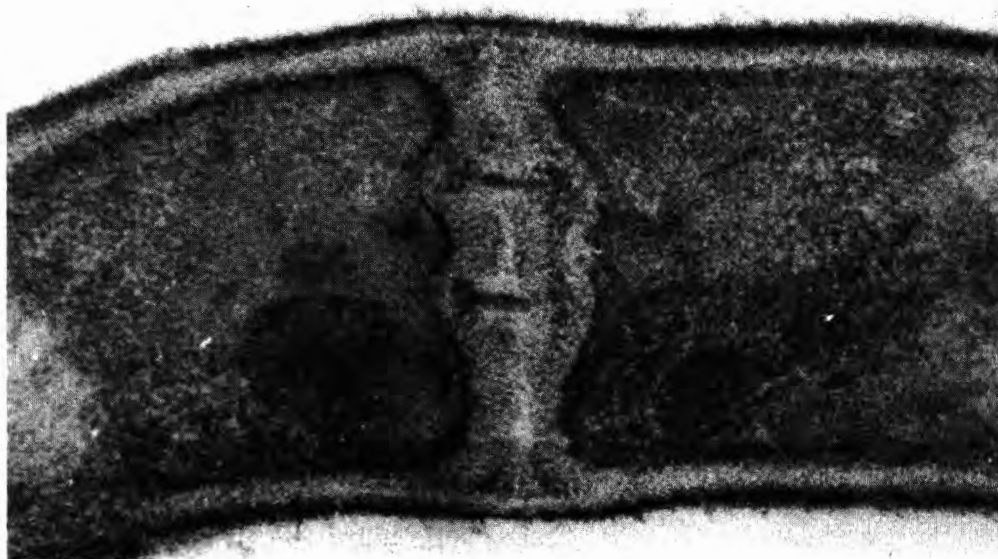
Adenine aminohydrolase (EC 3.5.4.2) was assayed by determining the released ammonia with Nessler's reagent. Cell-free extracts were incubated with 0.1 M potassium phosphate buffer at pH 7.0 and 1 mM adenine. The enzyme was only detected when 50 mM reduced glutathione and 50 mM EDTA had been added to the cells prior to sonic disruption.

Electron microscopy

Yeast cells were harvested, washed with distilled water and subsequently fixed

Table 1. Physiological characteristics of *Trichosporon adeninovorans* sp. nov.

Fermentation	glucose	+ s	sucrose	+ s	lactose	—
	galactose	+ s	maltose	+ s	raffinose	+ s
Assimilation of carbon compounds	galactose	+	raffinose	+	<i>D</i> -erythritol	+
	sucrose	+	soluble starch	+	ribitol	+
	maltose	+	<i>D</i> -xylose	+	<i>D</i> -mannitol	+
	cellobiose	+	<i>L</i> -arabinose	+	succinic acid	+
	trehalose	+	<i>D</i> -ribose	+	citric acid	+
	lactose	+ s, w	<i>L</i> -rhamnose	+ w	<i>D</i> -inositol	+
Additional carbon compounds tested	galactitol	+ s	ethanol	+	decane	+
	melibiose	+	octane	+ s, w	hexadecane	+
	melezitose	+				
Additional characteristics	assimilation of nitrate	+	splitting of arbutin	+		
	growth in vitamin-free medium	—	sodium chloride tolerance	17.5% (w/v) in malt agar		
	growth on 50% and 60% (w/w) glucose		maximum temperature of growth	45°C		
	yeast extract agar	+	urease	—		

Fig. 2. Electron micrograph of *Trichosporon adeninovorans* TOA-3 showing a longitudinal section through a hypha with plasmodesmata in the septum. Magnification 62 500 $\times$.

in potassium permanganate ($15 \text{ g} \cdot \text{l}^{-1}$) 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.

DESCRIPTION

Trichosporon adeninovorans Middelhoven, Hoogkamer-te Niet et Kreger-van Rij sp. nov.

In extracto malti cellulae subovoideae aut longovoideae $(2.2\text{--}3.6) \times (2.4\text{--}5.6) \mu\text{m}$; pseudomycelium et mycelium verum cum blastosporis et arthrosporis. Sedimentum et pellicula formantur. Cultura in agar malti flavalbida, tenax aut non tenax, crispulata. Plasmodesmata in septis. Endosporeae asexuales.

Glucosum, galactosum, sucrosum, maltosum et raffinsum lente fermentantur. Glucosum, galactosum, sucrosum, maltosum, cellobiosum, trehalosum, lactosum (lente, exigue), raffinsum, melibiosum, melezitiosum, amyllum solibule, D-xylosum, L-arabiosum, D-ribosum, L-rhamnosum (exigues), erythritolum, ribitolum, D-mannitolum, galactitolum (lente), acidum succinicum, acidum citricum et inositolum assimilantur. Kalii nitras assimilatur. Arbutinum finditur. Ad crescentiam vitaminarum externarum necessariae sunt. Crescit in 45°C . Ureum non finditur.

Typus: TOA-3 (CBS 8244) isolatus ex terra Wageningen in collectione zymotica Centraalbureau voor Schimmelcultures, Delphis Batavorum.

Growth in malt extract: After 3 days at 25°C budding yeast cells are short-oval to long-oval, $(2.2\text{--}3.6) \times (2.4\text{--}5.6) \mu\text{m}$; branched, septate hyphae and arthrospores are also present. The hyphae have a diameter of $2.4 \mu\text{m}$. A sediment and a white, dry pellicle are formed.

Growth on malt agar: After 3 days at 30°C budding yeast cells, pseudomycelium, true hyphae and arthrospores are present. After one month at 20°C the streak culture is yellowish-white, partly soft and partly tough. The edge is fringed with mycelium.

Slide cultures on potato and corn meal agar: True, branched, septate mycelium is abundantly formed. It may split up into arthrospores. Spherical or short-oval blastospores occur in chains, occasionally branched, sometimes on short elevations on the hyphae, generally between, but not at the septa. Aerial hyphae occur.

Formation of asexual spores: Spherical to oval endospores of variable size and number (3–6) are observed in hyphal cells on Difco malt agar.

Formation of ascospores: Not observed.

Physiological characteristics: Fermentation and assimilation of various carbon compounds as well as some additional characteristics are summarized in Table 1. Adenine, uric acid, propylamine, butylamine, pentylamine, hexylamine, octylamine and putrescine are utilized as sole carbon and nitrogen source.

The ultrastructure of the cell wall is ascomycetous. The septa in the hyphae contain plasmodesmata in a random distribution (Fig. 2).

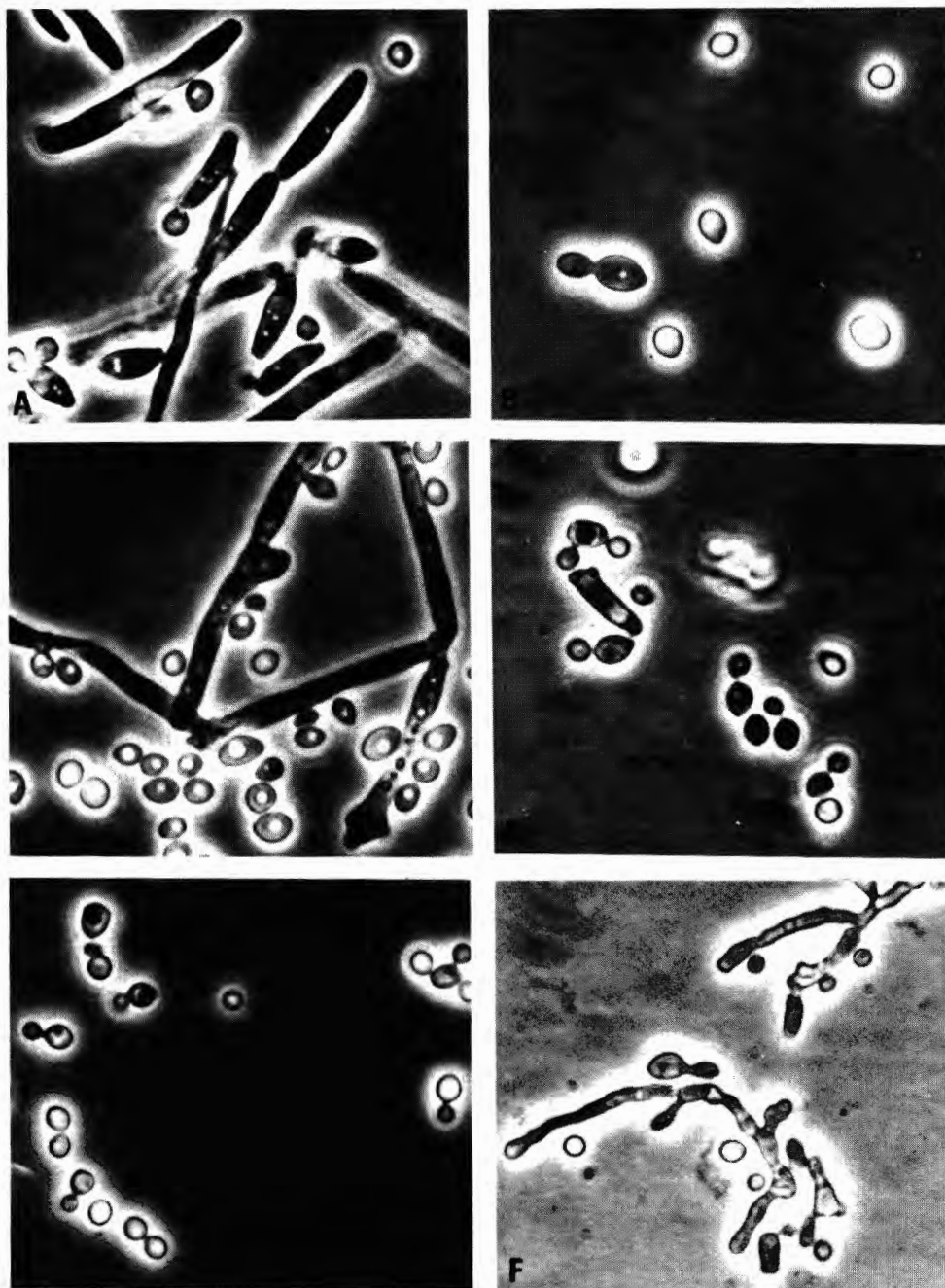


Fig. 3. Micrographs of *Trichosporon adeninovorans* TOA-3 and *Trichosporon terrestre* CBS 6697. Magnification 1 250 $\times$.

- A. TOA-3, 24 h, 30°C, malt agar slant; D. TOA-3, 3 days, 30°C, liquid uric acid medium;
 B. TOA-3, 24 h, 30°C, malt agar slant; E. TOA-3, 3 days, 30°C, liquid adenine medium;
 C. TOA-3, 4 weeks, 30°C, malt agar slant; F. CBS 6697, 4 days, 30°C, liquid uric acid medium.

Type strain: TOA-3 (CBS 8244), isolated from soil. This strain is maintained in the Yeast Division of the Centraalbureau voor Schimmelcultures at Delft.

RESULTS AND DISCUSSION

Tr. adeninovorans which is morphologically characterized by the formation of loose budding cells and true mycelium splitting up into arthrospores (Figs 3A, B, C) fits the diagnosis of the genus *Trichosporon* (Diddens and Lodder, 1942; Do Carmo-Sousa, 1970). Based on the ultrastructure of the cell wall (Figs 2 and 4), the species belongs to the ascomycetous group in this genus (Kreger-Van Rij and Veenhuis, 1971). During growth in liquid basal growth medium with adenine or uric acid mycelium was almost absent (Figs 3D, E).

Tr. adeninovorans is conspicuous because of the utilization of nitrate. The only other ascomycetous nitrate-positive species in this genus is *Trichosporon terrestre* van der Walt et Johannsen (1975). Like *Tr. adeninovorans*, *Tr. terrestre* can grow on adenine (very slowly), uric acid, butylamine, pentylamine and putrescine as sole source of carbon and nitrogen and the septa in the hyphae of this species contain plasmodesmata. However, *Tr. terrestre*, in contrast to *Tr. adeninovorans*, has no fermentative ability and does not assimilate soluble starch, melibiose and melezitose, propylamine and hexylamine. *Tr. terrestre* showed a strong tendency to mycelial growth, e.g. in liquid uric acid basal growth medium (Fig. 3F).

Growth on unusual carbon sources

Apart from a number of sugars and organic acids which are frequently utilized by yeasts, *Tr. adeninovorans* TOA-3 utilized purine compounds and amines as the sole source of carbon, nitrogen and energy. Growth on adenine was slow: a 1% inoculum pregrown in the same medium needed about 4 days at 30°C to attain the stationary growth phase. The specific growth rate was roughly estimated to be about 0.05 h^{-1} . Growth on adenine could be enhanced by raising the growth temperature to 37°C. Specific growth rates on uric acid were about 0.1 h^{-1} at 30°C and 0.15 h^{-1} at 37°C. Growth on adenine was maximal at pH 5.0; growth on uric acid was not influenced by the pH in the range of pH 5.0 to 7.0. Growth on adenine or uric acid was not markedly influenced by the salt concentration: growth rates in basal growth media with $1 \text{ g} \cdot \text{l}^{-1}$ or $20 \text{ g} \cdot \text{l}^{-1}$ KH_2PO_4 were about the same. Growth on xanthine, however, was observed only at pH 6.5 to 7.0 in basal growth medium with a high salt concentration (10 or $20 \text{ g} \text{ KH}_2\text{PO}_4 \cdot \text{l}^{-1}$). Strain TOA-3 also grew with guanine and 6,8-dihydroxypurine as the sole carbon source but not with isoguanine, 8-hydroxyadenine, 2,8-dihydroxyadenine or allantoin.

Growth yields as measured by determination of the dry weight of cultures when the purine substrate had been depleted, varied considerably with the sub-

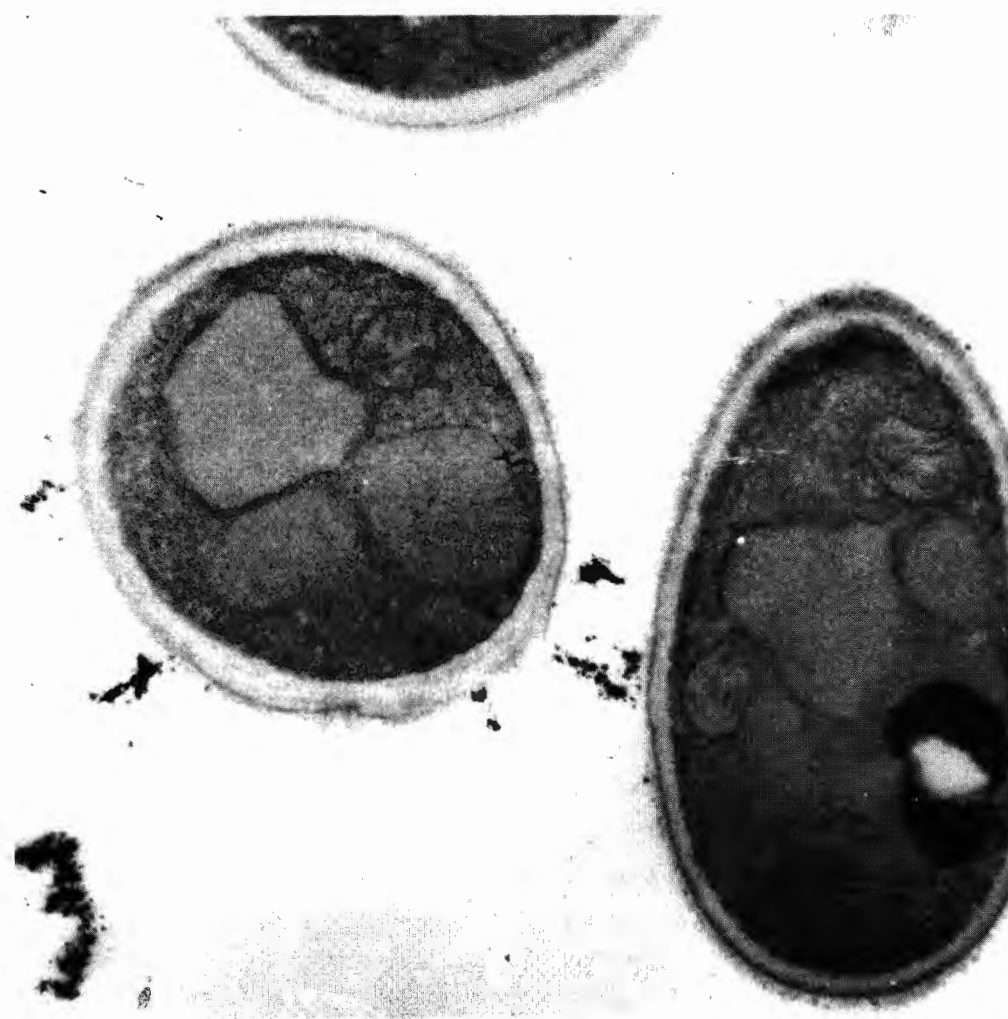


Fig. 4. Electron micrograph of a thin section of *Trichosporon adeninovorans* TOA-3 grown in liquid basal growth medium with adenine. The cells contain many large peroxisomes. Magnification 62500 $\times$.

Table 2. Specific enzyme activities of TOA-3 cells grown with substituted purine compounds as the sole source of carbon and energy ($\mu\text{mol} \cdot \text{h}^{-1} \text{mg protein}^{-1}$)

Carbon source	Urate oxidase	Allantoinase	Catalase
Adenine	8	30.4	430
Xanthine	2	n.d. ¹	135
Uric acid	17	8.1	340

¹n.d. not determined.

strate used. On uric acid 0.06 g dry biomass was produced per g ($10.1 \text{ g} \cdot \text{mol}^{-1}$), on xanthine 0.109 g per g ($16.6 \text{ g} \cdot \text{mol}^{-1}$) and on adenine 0.133 g per g ($18.0 \text{ g} \cdot \text{mol}^{-1}$).

Tr. adeninovorans TOA-3 utilized putrescine, propylamine, butylamine, pentylamine, hexylamine and octylamine as the sole source of carbon, nitrogen and energy (in basal growth medium), but not ethylamine and cadaverine. Growth at the expense of *n*-hexadecane was very fast. Decane was also assimilated, but growth on octane was slow and weak.

Catabolism of adenine

In most of the purine-degrading aerobic organisms adenine is deaminated and subsequently dehydrogenated to uric acid which is oxidized to allantoin. The latter compound is hydrolysed in three successive steps to glyoxylate and urea (Vogels and Van der Drift, 1976). Purine-grown cells of strain TOA-3 showed high activities of urate oxidase, allantoinase and catalase (Table 2). These data strongly suggest that urate oxidase and allantoinase are involved in the purine degradative pathway of *Tr. adeninovorans*. Growth on adenine was accompanied with the development of many large peroxisomes (Fig. 4). In other urate-assimilating yeasts these organelles are the site of urate oxidase and catalase (Middelhoven et al., 1983; Veenhuis et al., 1984).

The initial steps of adenine breakdown in strain TOA-3 were less clear. The release of ammonia from adenine could be demonstrated at pH 7 and 8 but the activity of the enzyme, adenine amidohydrolase, was very low. Moreover, the reaction did not proceed linearly with time. Aerobic incubation of adenine and several other amino- and hydroxypurines with cell-free extracts prepared from adenine-grown cells resulted in a total disappearance of UV-absorbing compounds within 20 h. Thus, the purines were slowly degraded by hydrolysis and oxidation in the absence of coenzymes and without conversion to nucleosides or nucleotides. Cell-free extracts of adenine- or xanthine-grown cells of strain TOA-3 showed minute oxidase activities with hypoxanthine, xanthine and 8-hydroxyadenine ($0.5 \mu\text{mol} \cdot \text{h}^{-1} \text{ mg protein}^{-1}$). It is improbable that these activities play a significant part *in vivo*. Cell yields on xanthine and adenine were much higher than on uric acid (see above). This can only be explained by assuming energy conservation due to linkage of purine dehydrogenation to the electron transport chain.

Cell-free extracts of adenine- or xanthine-grown cells of strain TOA-3 catalysed the production of NADH from NAD^+ and several purine compounds, e.g. adenine, hypoxanthine, xanthine, 6,8-dihydroxypurine, guanine and isoguanine. Specific enzyme activities usually were 2 to $3 \mu\text{mol} \cdot \text{h}^{-1} \text{ mg protein}^{-1}$. All of the purine dehydrogenase and oxidase activities, except for urate oxidase, were strongly inhibited by 0.1 mM allopurinol and by 0.01 mM 8-phenylhypoxanthine. Allopurinol is a well-known inhibitor of xanthine dehydrogenases and xanthine oxidases from several sources. 8-Phenylhypoxanthine

is a more potent inhibitor of these enzymes (Bergmann et al., 1977). These inhibition studies indicate that all purine oxidase and purine dehydrogenase activities of *Tr. adeninovorans* TOA-3 should be ascribed to one enzyme complex which is closely related to xanthine dehydrogenase from animal and vegetable sources.

The data available suggest that adenine is deaminated to hypoxanthine which subsequently is dehydrogenated via xanthine or 6,8-dihydroxypurine to uric acid. Dehydrogenation of adenine in vivo is improbable, since the possible reaction products, isoguanine and 8-hydroxyadenine, were very slowly deaminated by cell-free extracts and were no growth substrates for strain TOA-3.

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