SHORT COMMUNICATION

Occurrence of Cytochrome P-450 in Yeasts

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Cytochrome P-450 was measured in whole cell suspensions of various yeasts, with Saccharomyces cerevisiae and S. uvarum (formerly carlsbergensis) serving as positive control species. Eleven yeasts, when grown on a medium with glucose (50 g l⁻¹) as carbon and energy source, contained cytochrome P-450. These were the Ascomycotina Debaryomyces hansenii, Hansenula anomala, Kluyveromyces fragilis, Pichia fermentans, Saccharomyces bayanus, S. chevalieri, S. italicus and Schizosaccharomyces japonicus, and the Deuteromycotina Brettanomyces anomalus, Torulopsis dattila and T. glabrata. The amount of cytochrome P-450 varied 60- to 70-fold between the species. The position of the Soret band in reduced CO spectra also differed from one species to another. Cytochrome P-450 could not be detected in either Candida utilis or a Rhodotorula sp.

INTRODUCTION

Hydroxylation of many compounds, for example, environmental chemicals, is catalysed by an enzyme system which contains cytochrome P-450 (Mannering, 1971; Daly et al., 1972). This mixed function oxidase system is found in animals, plants and fungi (Hodgson, 1974). However, only about 16 micro-organisms have been shown to contain cytochrome P-450 (for reviews, see Wiseman, 1977; Schunck et al., 1978). Among yeasts, this haemoprotein has been found in Candida tropicalis, C. guilliermondii, Endomycopsis lipolytica, Saccharomyces cerevisiae, S. carlsbergensis and Schizosaccharomyces pombe. It has also been suggested that it occurs in Torulopsis. Hydroxylation of foreign chemicals by this enzyme system was found in C. tropicalis (Duppel et al., 1973) and S. cerevisiae (Wiseman et al., 1975b; Woods & Wiseman, 1979). The aim of the present study was to broaden the range of micro-organisms studied for cytochrome P-450 content. Yeasts were chosen as test organisms, because they are eukaryotic and, since they do not form mycelium, they are easy to cultivate and study under different conditions.

METHODS

Yeast species and strains. The following were used: Brettanomyces anomalus C01, Candida tropicalis (Castellani) Berkhout C14, C. utilis C15, Debaryomyces hansenii C20, Hansenula anomala C21, Kluyveromyces fragilis (formerly S. fragilis) C25, Pichia fermentans C31, Saccharomyces bayanus (formerly S. pastorianus, formerly S. oviformis) C36, S. cerevisiae HansenNCYC 1200 andNCYC 240 (brewer's yeasts), S. cerevisiae Hansen B02 and B10 (baker's yeasts), S. chevalieri C57, S. italicus C63, S. uvarum Beijerinck (formerly S. carlsbergensis Hansen) A07, A15, A25, A27 and A50, Schizosaccharomyces japonicus var. versatilis C70, Torulopsis dattila C72 and T. glabrata C74, from the Technical Research Center, Finland; Rhodotorula sp.,
isolated from air samples and identified at the Department of Environmental Hygiene, University of Kuopio, Finland.

**Maintenance and growth of cultures.** Stock cultures of the yeasts were maintained on agar slopes which contained the same substances as the respective liquid media plus agar (20 g l⁻¹). The liquid growth medium for *Rhodotorula* sp. contained (g l⁻¹): KH₂PO₄, 1; MgSO₄, 7H₂O, 0.5; NH₄Cl, 0.5; FeCl₃, 0.01; malt extract, 5; d-glucose, 20. The pH was adjusted to 7.0 before autoclaving. Other strains were grown on a medium described by Wiseman et al. (1975a). Glucose (50 g l⁻¹) was sterilized separately. Alkane medium for *C. tropicalis* and *C. utilis* was described by Duppen et al. (1973).

Growth medium (250 ml) in a 500 ml bottle plugged with cotton wool was inoculated and incubated at 30°C in a shaker. Samples were removed at different times (19 to 120 h) and centrifuged at 2500 g for 5 min; the pellets were frozen at −20°C. Growth was followed turbidimetrically at 600 nm, and wet weight of yeast was determined by reference to a standard calibration curve prepared for *S. cerevisiae*.

**Measurement of cytochrome P-450.** Cytochrome P-450 was measured principally as described by Omura & Sato (1964). The cells were thawed, 0.6 g wet wt was suspended in 6 ml 0·1 M-KH₂PO₄/KOH buffer, pH 7·4, and a few mg of sodium dithionite were added to complete the reduction of cytochrome P-450. Carbon monoxide (prepared from formic acid by dropwise addition of concentrated H₂SO₄) was bubbled through the sample cuvette. The CO difference spectra were recorded at room temperature with an automatically scanning Cary model 118 (Varian) spectrophotometer. The height of the peak was measured at the wavelength of maximum absorbance near 450 nm. The approximate contents of cytochrome P-450 were calculated assuming that the absorptivity for the difference in absorption of the haemoprotein between about 450 and 500 nm is 92.1 mmol⁻¹ cm⁻¹ (Yoshida et al., 1977).

**RESULTS AND DISCUSSION**

In the present study, 14 of 16 yeasts tested contained a cytochrome P-450-like haemoprotein (Table 1); 11 of these 14 have not previously been observed to contain this entity. This haemoprotein had a Soret band near 450 nm in the CO-reduced state (Fig. 1). As a control, cytochrome P-450 was measured simultaneously in *S. cerevisiae* and *S. uvarum*, which are known to contain this haemoprotein (Yoshida et al., 1977; Cartledge et al., 1972). *Candida utilis* and *Rhodotorula* sp., when grown on a glucose-containing medium, did not show any peak around 450 nm in the reduced CO-difference spectra. The same was also true for *C. tropicalis*, which agrees with the results of Gallo et al. (1976). *Candida utilis*, when cultured on alkane medium, on which it grew slowly, still did not contain any cytochrome P-450, but *C. tropicalis*, under similar growth conditions, did contain the cytochrome (see also Gallo et al., 1976).

The yeasts studied belong to two subdivisions of fungi, namely Ascomycotina and Deuteromycotina (imperfect fungi) (Ainsworth et al., 1973). All Ascomycotina studied belong to the family Saccharomycetacea (Debaryomyces, Hansenula, Kluyveromyces, Pichia, Saccharomyces, Schizosaccharomyces). All species and strains studied from these genera so far contain cytochrome P-450. In addition, *Endomycopsis*, which is also known to contain cytochrome P-450 (Delaisse & Nyns, 1974), belongs to the Saccharomycetacea. All Deuteromycotina studied belong to the family Cryptococcaceae (Brettanomyces, Candida, Rhodotorula, Torulopsis). This family includes genera or species which at least under certain growth conditions do not contain cytochrome P-450. *Candida* is an example of a genus with species which differ with respect to the occurrence of cytochrome P-450. Among bacteria a similar example would be *Pseudomonas* (Broadbent & Cartwright, 1974). It has been suggested (Callen & Philpot, 1977) that the ‘Crabtree effect’ might have some regulatory role in the biosynthesis of cytochrome P-450 in yeast. The present experiment indicated that both Crabtree-positive (e.g. *S. chevalieri*, *S. italicus*, *S. bayanus*, *T. dattila*, *T. glabrata*) and Crabtree-negative (e.g. *K. fragilis*, *H. anomala*, *P. fermentans*) species (DeDeken, 1966) contained cytochrome P-450 under the growth conditions used.

The amount of the haemoprotein in the yeasts (Table 1) varied considerably during different growth stages (Fig. 1).

Some different strains of *S. cerevisiae* and *S. uvarum* were also studied. No differences were observed among the various strains of *S. cerevisiae*. Among individual strains of
Table 1. Occurrence of cytochrome P-450 in some yeasts

Values represent the highest concentrations observed in parallel cultures recorded at the growth times indicated for each species.

<table>
<thead>
<tr>
<th>Yeast</th>
<th>Growth time</th>
<th>( \lambda_{\text{max}} )</th>
<th>Cytochrome P-450</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brettanomyces anomalus</td>
<td>43.5 h</td>
<td>449 nm</td>
<td>7.82 nmol (g wet wt)(^{-1} )</td>
</tr>
<tr>
<td>Candida tropicalis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose-grown</td>
<td>48 h</td>
<td>---</td>
<td>0 nmol (g wet wt)(^{-1} )</td>
</tr>
<tr>
<td>Alkane-grown</td>
<td>96 h</td>
<td>453 nm</td>
<td>5.11 nmol (g wet wt)(^{-1} )</td>
</tr>
<tr>
<td>Candida utilis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose-grown</td>
<td>48 h</td>
<td>---</td>
<td>0 nmol (g wet wt)(^{-1} )</td>
</tr>
<tr>
<td>Alkane-grown</td>
<td>117 h</td>
<td>---</td>
<td>0 nmol (g wet wt)(^{-1} )</td>
</tr>
<tr>
<td>Debaryomyces hansenii</td>
<td>43.5 h</td>
<td>456 nm</td>
<td>0.13 nmol (g wet wt)(^{-1} )</td>
</tr>
<tr>
<td>Hansenula anomala</td>
<td>19 h</td>
<td>450 nm</td>
<td>4.07 nmol (g wet wt)(^{-1} )</td>
</tr>
<tr>
<td>Kluyveromyces fragilis</td>
<td>19 h</td>
<td>451 nm</td>
<td>1.87 nmol (g wet wt)(^{-1} )</td>
</tr>
<tr>
<td>Pichia fermentans</td>
<td>19 h</td>
<td>454 nm</td>
<td>0.95 nmol (g wet wt)(^{-1} )</td>
</tr>
<tr>
<td>Rhodotorula sp.</td>
<td>96 h</td>
<td>---</td>
<td>0 nmol (g wet wt)(^{-1} )</td>
</tr>
<tr>
<td>Saccharomyces bayanus</td>
<td>19 h</td>
<td>448 nm</td>
<td>3.68 nmol (g wet wt)(^{-1} )</td>
</tr>
<tr>
<td>S. cerevisiae NCYC 240</td>
<td>41</td>
<td>448 nm</td>
<td>4.66 nmol (g wet wt)(^{-1} )</td>
</tr>
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<td>S. chevalieri</td>
<td>19</td>
<td>448 nm</td>
<td>1.62 nmol (g wet wt)(^{-1} )</td>
</tr>
<tr>
<td>S. italicus</td>
<td>19</td>
<td>449 nm</td>
<td>9.07 nmol (g wet wt)(^{-1} )</td>
</tr>
<tr>
<td>S. uvarum A15*</td>
<td>48</td>
<td>448 nm</td>
<td>2.51 nmol (g wet wt)(^{-1} )</td>
</tr>
<tr>
<td>Schizosaccharomyces japonicus</td>
<td>43.5 h</td>
<td>447 nm</td>
<td>1.11 nmol (g wet wt)(^{-1} )</td>
</tr>
<tr>
<td>Toruloepis dattila</td>
<td>19</td>
<td>447 nm</td>
<td>3.95 nmol (g wet wt)(^{-1} )</td>
</tr>
<tr>
<td>T. glabrata</td>
<td>19</td>
<td>448 nm</td>
<td>6.22 nmol (g wet wt)(^{-1} )</td>
</tr>
</tbody>
</table>

* Grown without shaking or other type of aeration in medium containing (g l\(^{-1} \)): malt extract, 3; yeast extract, 3; peptone, 5; D-glucose, 200.

S. uvarum about threefold differences were found, strains A15, A25 and A27 having the highest contents of cytochrome P-450. The amount of cytochrome P-450 observed in S. cerevisiae agrees with the results of Wiseman et al. (1975a).

The position of the apparent maximum of the Soret band for cytochrome P-450 in the reduced CO-difference spectra varied among different yeast species (Table 1, Fig. 1a).

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**Fig. 1.** Reduced CO-difference spectra of some yeasts. (a) Late-exponential growth phase: 1, B. anomalus, growth time 43.5 h (absorbance scale A\(_2\)); 2, T. dattila, 19 h (A\(_3\)). (b) Stationary growth phase: 1, B. anomalus, 90.5 h (A\(_2\)); 2, H. anomala, 43.5 h (A\(_3\)); 3, S. chevalieri, 43.5 h (A\(_3\)).
Possibly there are different kinds of cytochrome P-450 in different yeast species. It is well known that in mammals two spectrally different forms of cytochrome P-450, namely P-450 and P-448, can be distinguished (Mannering, 1971). The location of the wavelength maximum appeared to depend also on the growth stage of the yeast. The peak of cytochrome P-450 in the reduced CO-difference spectrum moved to a higher wavelength as the culture aged, and the cytochrome P-450 content diminished. The different physiological states during different growth stages could cause this through different types and amounts of CO-binding pigments is required.

In addition to cytochrome P-450, the reduced CO-difference spectra recorded from whole yeast cells contained other peaks in the wavelength region of 400 to 500 nm. At least three peaks or shoulders could be observed at about 413, 421 and 428 nm (Fig. 1). Since these peaks increased when the cytochrome P-450 content decreased, they could be partly derived from other CO-binding pigments.


The physiological role of cytochrome P-450 is unknown in the new yeast species studied in this report. Preliminary results from this laboratory show that K. fragilis and T. glabrum can hydroxylate biphenyl in the presence of cumene hydroperoxide. If these haemoproteins have the same catalytic abilities as mammalian cytochrome P-450 isoenzymes, yeasts could serve as useful models for studies on the metabolism of xenobiotics.

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