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* Hansen NCYC 1200 and NCYC 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 Duppel *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 l 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 Saccharomycetaceae (*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 (Delaissé & Nyns, 1974), belongs to the Saccharomycetaceae. 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.

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

* 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.

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_1). (b) Stationary growth phase: 1, *B. anomalus*, 90.5 h (A_2); 2, *H. anomala*, 43.5 h (A_1); 3, *S. chevalieri*, 43.5 h (A_1).

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).

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. The largest effect might be expected to derive from cytochrome a_3 which causes a negative peak at around 440 nm in the reduced CO spectrum (Lindenmayer & Smith, 1964; Ishidate *et al.*, 1969). To study this further, purification of cytochrome P-450 from other 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*b*). Since these peaks increased when the cytochrome P-450 content decreased, they could be partly derived from cytochrome P-450. In addition, cytochrome a_3 , protohaem, cytochrome *c* peroxidase, haemoglobin or catalase may contribute to the peaks (Lindenmayer & Smith, 1964; Ishidate *et al.*, 1969).

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. glabrata* 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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