Cytochrome P-450 Accumulation and Loss as Controlled by Growth Phase of *Saccharomyces cerevisiae*: Relationship to Oxygen, Glucose and Ethanol Concentrations

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Ethanol induced small amounts of cytochrome P-450 in *Saccharomyces cerevisiae* NCYC 754 under conditions in which it is not normally detectable. Moreover, in non-growing yeast the existing cytochrome P-450 content was increased by 50% at a limited range of glucose concentrations (8–12% in 0.1 M-potassium phosphate buffer, pH 7.0), in which ethanol is produced by fermentation, possibly at an optimum concentration for induction of cytochrome P-450. Added alkanols, other than ethanol, caused rapid degradation of cytochrome P-450 in non-growing yeast; the rate of loss was directly related to the lipid solubility of the alkanol. Ethanol therefore favoured the accumulation of cytochrome P-450 in yeast; this may be related to an important putative role of one of the isoenzymes in ethanol-tolerance of the yeast, by the oxidative removal of ethanol from the endoplasmic reticulum of the cell. It is the accumulation of dissolved oxygen, rather than ethanol, that occurs on cessation of yeast growth that is likely to trigger the rapid disappearance of cytochrome P-450 observed at this time.

INTRODUCTION

Cytochrome P-450 enzymes [RH, reduced-flavoprotein:oxygen oxidoreductase (RH-hydroxylating), EC 1.14.14.1] are a group of haemoproteins which catalyse the mono-oxygenation of both endogenous and exogenous substrates. In *Saccharomyces cerevisiae*, cytochrome P-450 is involved in the 14 α -demethylation of lanosterol during the biosynthesis of ergosterol, which is required for yeast membrane synthesis in the endoplasmic reticulum (Aoyama *et al.*, 1984). Cytochrome P-450 from *S. cerevisiae* can also catalyse the mono-oxygenation of some xenobiotic compounds such as benzo(a)pyrene (Wiseman & Woods, 1979; King *et al.*, 1984), aminopyrine, *p*-nitroanisole and caffeine (Sauer *et al.*, 1982), and activate many promutagens to their active mutagenic products (Callen *et al.*, 1980; Kelly & Parry, 1983).

During growth under strict fermentation conditions, i.e. at glucose concentrations in the range 5–20% (w/v), cells of *S. cerevisiae* accumulate large amounts of cytochromes P-450 – more than is apparently required for its endogenous role in the 14 α -demethylation of lanosterol (Aoyama *et al.*, 1981). The reasons for this accumulation of cytochromes P-450 are not well understood, although factors such as glucose, oxygen and ethanol are believed to be important (Blatiak *et al.*, 1985*a*; Wiseman *et al.*, 1978; Morita & Mifuchi, 1984; Del Carratore *et al.*, 1984). Also, under these conditions, glucose repression of mitochondrial proteins, such as cytochrome *a* + *a*₃, occurs, and the yeast therefore derives its energy by fermentation.

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The production of cytochrome P-450 in *S. cerevisiae* was shown by tetrad analysis to be controlled by a single nuclear gene which was not the structural gene, with at least one modifier gene being involved in the modulation of the amount of cytochrome P-450 produced (King *et al.*, 1983). The identity of these genes is so far unknown. Cyclic AMP may be involved in regulating cytochrome P-450 production in *S. cerevisiae* by some form of negative control (Wiseman *et al.*, 1978). In addition, cytochrome P-450 may be destroyed in stationary phase culture by a cyclic-AMP-dependent covalent modification, as reported for the enzyme from rabbit liver (Muller *et al.*, 1985), which could be oxygen-concentration dependent. Alternatively, oxygen or oxygen-radicals could inactivate the enzyme by directly binding to it.

It has been shown that the production of the yeast haemoproteins iso-1-cytochrome *c*, catalase T and catalase A is co-ordinately controlled by glucose, oxygen and haem through the control of mRNA levels (Hortner *et al.*, 1982), although there is also regulation at the post-transcriptional level (Laz *et al.*, 1984). The amount of iso-1-cytochrome *c* and iso-2-cytochrome *c* is reduced in yeast grown anaerobically or under glucose repression, whereas the amount of apoprotein of each of cytochrome *b*, cytochrome *c* peroxidase and cytochrome *c* is not reduced (Ross & Schatz, 1976). The production and addition of the haem is therefore the crucial controlling step in these systems, although it should be noted that biosynthesis of the haem moiety occurs in mitochondria (Gudenus *et al.*, 1984). Cytochrome P-450 accumulates in yeast endoplasmic reticulum and is detectable spectrally when yeast is grown semi-anaerobically or under conditions of glucose repression. Different haemoproteins respond differently to glucose, oxygen, haem and ethanol; therefore, studies on the regulation of these systems should contribute to our understanding of the mechanisms involved in the co-ordinate control of the production of eukaryotic proteins.

We have recently shown that cytochrome P-450 is induced in *S. cerevisiae* by small amounts of oxygen, presumably by removal of blocking mechanisms at the genome level, but that at high oxygen concentrations the cytochrome P-450 content is reduced (Blatiak *et al.*, 1985*a*). In this study we report on the roles of ethanol and glucose in cytochrome P-450 accumulation, and relate this to oxygenation, both during growth and in stationary phase culture.

METHODS

Organism and growth. *S. cerevisiae* NCYC 754 was maintained on slopes of Sabouraud dextrose agar. Liquid cultures were grown aerobically at 30°C for up to 180 h in 100 ml medium in a 250 ml conical flask shaken at 120 r.p.m. in an orbital shaker. The medium contained the following (% w/v): D-glucose (20); mycological peptone (2); yeast extract (1); and NaCl (0.5).

Yeast cells, harvested in late exponential phase of growth (35–40 h), were washed and resuspended in 0.1 M-potassium phosphate buffer, pH 7.0. Members of the n-alkanol series (6%, v/v) were added in separate experiments, and incubated with shaking in a sealed flask (to avoid evaporation of alkanol) at 30°C for several hours. Samples were withdrawn at intervals for assay of residual cytochrome P-450.

Assay methods. Cytochrome P-450 was determined directly on whole yeast cells by the reduced carbon monoxide difference spectrum method of Omura & Sato (1964), using an extinction coefficient of 91 mm⁻¹ cm⁻¹. Ethanol was measured enzymically by using alcohol dehydrogenase and measuring the increase in absorbance at 340 nm of NADH, using a kit from Boehringer. Glucose was measured by the glucose oxidase method also using a kit from Boehringer.

Batch fermenter growth. Some experiments, as noted later, were done in a 4 l microprocessor controlled batch fermenter under optimal conditions for cytochromes P-450 production. These were as follows: 25.1°C, pH 5.04, impeller speed 253 r.p.m., gas flow rate 150 ml min⁻¹, in a medium containing (% w/v) glucose (14.05), mycological peptone (1.04), yeast extract (1.69) and NaCl (0.09). The conditions for the growth of yeast in the orbital shaker were also close to the optimum for oxygenation. These were chosen on the basis of previous work (Blatiak *et al.*, 1985*a*) so that the maximum growth rate of yeast, and the maximum accumulation of cytochrome P-450 in 44 h could be approached.

RESULTS AND DISCUSSION

The cytochrome P-450 content of cells of *S. cerevisiae* NCYC 754 was closely linked to the growth phase. It reached a maximum at the end of exponential growth, and fell rapidly soon after the onset of the stationary phase at about 40 h in medium containing 20% (w/v) glucose

Fig. 1

Fig. 2

Fig. 1. Production of cytochrome P-450 during yeast growth in 20% (w/v) glucose medium correlated with the rise in ethanol concentration and loss of glucose in the growth medium. ●, Cytochrome P-450; ■, glucose; ▲, ethanol. Values are the means of six determinations; error bars indicate SD.

Fig. 2. Effect of ethanol on cytochrome P-450 loss. Yeast cells were resuspended and incubated aerobically with shaking in 100 mM-potassium phosphate buffer, pH 7.0, containing no ethanol (○) or 6% (v/v) ethanol (●). Values are the means of six determinations; error bars indicate SD.

Table 1. *Half-life of cytochrome P-450*

Yeast cells were incubated at 30 °C in the presence (6%, v/v) of the first five members of the n-alkanol series. Results are the means of two independent determinations (which did not differ significantly).

Alkanol	Half-life (min)
None*	420
Methanol	130
Ethanol	510
Propanol	100
Butanol	20
Pentanol	2.5

* Potassium phosphate buffer only.

(Fig. 1). The half-life of the enzyme was about 20 h in this residual medium containing about 4% (w/v) glucose and 5% (v/v) ethanol, as distinct from 7 h in potassium phosphate buffer (Table 1). As expected, the glucose concentration of the medium declined rapidly with a concurrent increase in ethanol concentration which later fell, due to evaporation losses and the probable use of ethanol as a carbon source under these aerobic, low-glucose conditions. The rapid fall of cytochrome P-450 content in stationary phase has been partially attributed to the decline in the glucose concentration in the growth medium (Wiseman *et al.*, 1975). Another possible factor is the rise in ethanol concentration to approximately 6% (v/v) at the end of exponential growth. Our results do not substantiate this view, but imply that loss of cytochrome P-450 is due to buildup of oxygen in the medium after growth ceases, because the yeast is no longer rapidly removing it, resulting in oxygen, or active-oxygen, mediated destruction of the enzyme.

Until recently, it was thought that yeast accumulated ethanol intracellularly to a concentration higher than that in the medium during fermentation (Beaver *et al.*, 1982; Loureiro & Ferreira, 1983). However, recent work suggests that *S. cerevisiae* does not accumulate ethanol against a concentration gradient but that the yeast plasma membrane is freely permeable to ethanol (Guijarro & Lagunas, 1984). Ethanol (and other alkanols) are known to have many deleterious effects on yeast at higher concentrations, including an inhibitory effect on growth rate, fermentation rate and viability (Brown *et al.*, 1981; Jones & Greenfield, 1985). The mechanisms of these effects are unknown, although contributory factors might be the reduction in the uptake of solutes such as glucose (Leao & Van Uden, 1982*a, b*) and amino acids (Leao & Van Uden, 1984*a*), passive proton influx across the plasma membrane (Leao & Van Uden,

1984*b*) and thermal death (Leao & Van Uden, 1982). Little is known about the effects of ethanol on the synthesis and degradation of enzymes in stationary phase yeast, although it should not be overlooked that ethanol causes a decrease in water activity and in dielectric constant of the growth medium. The decrease in viability caused by ethanol may be initiated by the interaction of ethanol with lipid components of cell membranes as suggested by Thomas *et al.* (1978), who demonstrated that yeast cells with added ergosterol were more resistant to ethanol. Casey *et al.* (1983) showed that supplementation with ergosterol and Tween 80 enabled yeast to grow in much higher concentrations of ethanol. Yeast cytochrome P-450 is a key enzyme for ergosterol biosynthesis (Aoyama *et al.*, 1984), so if ethanol were affecting sterol biosynthesis through an effect on cytochrome P-450, then ergosterol supplementation would indeed be expected to overcome this. However, recent work has suggested that supplementation with sterols including ergosterol could not protect yeast from ethanol inhibition of growth (Walker-Caprioglio *et al.*, 1985). Nevertheless, yeast is particularly sensitive to inhibition of growth and fermentation by ethanol under anaerobic conditions (Aguilera & Benitez, 1985), presumably when cytochrome P-450 is absent.

The effect of added ethanol on the cytochrome P-450 content of late exponential phase yeast cells was examined. In control incubations in potassium phosphate buffer the cytochrome P-450 content declined in 7 h to approximately 45% of that present originally. When ethanol was added little if any change in degradation rate was seen at first, but after 7 h the cytochrome P-450 content had stabilized to approximately 65% of that present originally. This may reflect an altered degradation rate after 4–5 h, possibly with additional synthesis of ethanol-induced cytochrome P-450. This effect is also reflected in an increased half-life for cytochrome P-450 when yeast cells were incubated with ethanol compared to the control in buffer alone (Table 1). This effect was not seen with alkanols other than ethanol, all of which accelerated degradation: with increasing chain length of alkanol there was a faster loss of cytochrome P-450 (Table 1). This finding reflects the lipid-solubility of the alkanols: the more lipid-soluble the alkanol, the faster the degradation caused by its addition. The lipid-solubility of alkanols also correlates with many other effects of these compounds on yeast such as on glucose transport and fermentation (Leao & Van Uden 1985), ammonium transport (Leao & Van Uden, 1983) and thermal death (Leao & Van Uden, 1982*a, b*). This suggests that alkanols interfere with hydrophobic membrane regions, including the endoplasmic reticulum in which cytochrome P-450 is located. Ethanol, however, seems exceptional in its lack of destructive effect on this enzyme.

In yeast grown in medium containing 0.5% glucose, cytochrome P-450 could not be detected by spectral means (King, 1982); this finding is supported by the work of Aoyama *et al.* (1981) and Karenlampi *et al.* (1981). It may be significant that about 35% more oxygen is dissolved in media containing about 0.5–1% glucose than in the media containing 20% glucose where P-450 is accumulated. Addition of ethanol to low-glucose media may overcome this effect. To further examine this, cultures of yeast in media containing 0.5% glucose were supplemented with 1% ethanol. This resulted in the induction of a small amount of cytochrome P-450 between 20 and 50 h of incubation (Fig. 3). Ethanol induction of cytochrome P-450 in *S. cerevisiae* was also observed by Del Carratore *et al.* (1984) and Morita & Mifuchi (1984). This induced form of cytochrome P-450 may be similar to that in mammalian liver, in which ethanol induces a form of P-450 which operates via a free-radical mediated mechanism to oxidize ethanol (Ingelman-Sundberg & Hagbjork, 1982). The metabolism of 8-methoxypsoralen by *S. cerevisiae* is also induced by ethanol (Prognon *et al.*, 1984), and this may be mediated by a cytochrome P-450 enzyme system. Ethanol is, of course, present in yeast during fermentative growth, under which conditions cytochrome P-450 (cytochrome P-448 isoenzyme) is produced in large quantities. It is likely, therefore, that under these conditions ethanol-induced cytochrome P-450 is synthesized in the endoplasmic reticulum of the yeast cell, and that the main role of this isoenzyme is to oxidize ethanol. Indeed, substrate binding of ethanol to this isoenzyme could stabilize it. The removal of ethanol would be expected to protect the protein biosynthesis system. It should be noted that such forms of cytochrome P-448, functioning by means of free radicals, would be sensitive to inhibition by free radical trapping agents such as the dithiothreitol often used to stabilize cytochromes P-450 during isolation and assay.

Fig. 3

Fig. 3. Production of cytochrome P-450 during yeast growth in 0.5% (w/v) glucose medium containing 1% (v/v) ethanol. In the absence of ethanol no cytochrome P-450 could be detected. Values are the means of six determinations; error bars indicate SD.

Fig. 4

Fig. 4. Effect of added glucose on the cytochrome P-450 content of yeast incubated aerobically with shaking in 100 mM-potassium phosphate buffer, pH 7.0, containing 0 (▼), 1 (▲), 8 (○) or 12% (●) (w/v) glucose. Values are the means of six determinations; error bars indicate SD.

High concentrations of glucose in the growth medium cause the accumulation of P-450 in growing yeast. In comparison, the effect of incubating yeast after transfer to non-growth conditions in potassium phosphate buffer, pH 7.0, with different concentrations of glucose added, is shown in Fig. 4. With 8% (w/v) glucose the already high content of cytochrome P-450 rose after 2–5 h to 150% of that present originally, but later fell. With 1% (w/v) glucose this effect was not seen, whereas with 5% (w/v) glucose some accumulation of P-450 occurred. At higher glucose concentrations less accumulation of P-450 was found, so that with 12 and 20% (w/v) glucose results were similar. Under these conditions fermentation of the glucose occurs, and it is possible that at 8–12% (w/v) glucose, fermentation may result in an optimum concentration of ethanol to further induce P-450. No such further accumulation of cytochrome P-450 could be observed using the 4 l microprocessor controlled fermenter. However, here the oxygenation may be high enough to destroy the enzyme or, alternatively, to prevent attainment of the optimum concentration of ethanol for induction. In earlier work we found that there was protection from degradation of P-450 by anaerobiosis and other agents that inhibited protein biosynthesis in yeast mitochondria. These would also stop the aerobic metabolism of ethanol (Blatiak *et al.*, 1980).

The large accumulation of P-450 in yeast during rapid fermentative growth on high-glucose media has long been a puzzle when it seems that sufficient P-450 for 14 α -demethylation of lanosterol can be made even aerobically in low-glucose media (Aoyama *et al.*, 1981). Although growth at a high glucose concentration is very much faster than that at a low glucose concentration, it is doubtful whether all of the extra P-450 produced would be needed for the production of sufficient ergosterol at this faster rate of growth. Therefore, the role of this high-glucose induced P-450 is not understood. The induction observed in high-glucose media might be related to the concentration of ethanol produced rather than the glucose concentration. In mammalian systems ethanol is known to induce a specific form of P-450 which has a very high activity towards the oxidation of ethanol to acetaldehyde (Coon *et al.*, 1984), and is also capable of oxidizing a range of other substrates by a free-radical mediated mechanism (Ingelman-Sundberg & Hagbjork, 1982). It is likely that in *S. cerevisiae* a comparable system is present, which is also capable of oxidizing some xenobiotics such as benzo(a)pyrene.

It is obvious that a complex mechanism of regulation of P-450 is present in *S. cerevisiae*. Low concentrations of oxygen induce P-450 whereas high concentrations repress or destroy the enzyme (Blatiak *et al.*, 1985a). We have now shown that ethanol, as well as glucose, is also involved in control of P-450 content. It is likely that haem made in mitochondria is also involved, as has been shown for iso-1-cytochrome *c* (Guarente & Mason, 1983), so that further

roles for oxygen are inevitable. The complexity of the regulatory system is perhaps not surprising, as several forms of cytochrome P-450 are now known to exist in *S. cerevisiae* (King *et al.*, 1982). Part of this work has been reported briefly in a preliminary communication (Blatiak *et al.*, 1985b).

REFERENCES

- AGUILERA, A. & BENITEZ, T. (1985). Role of mitochondria in ethanol tolerance of *Saccharomyces cerevisiae*. *Archives of Microbiology* **142**, 389–392.
- AOYAMA, Y., YOSHIDA, Y. & SATO, R. (1984). Yeast cytochrome P-450 catalysing lanosterol 14 α -demethylation. II. Lanosterol metabolism by purified P-450_{14DM} and by intact microsomes. *Journal of Biological Chemistry* **259**, 1661–1666.
- AOYAMA, Y., OKIKAWA, T. & YOSHIDA, Y. (1981). Evidence for the presence of cytochrome P-450 functional in lanosterol 14 α -demethylation in microsomes of aerobically grown respiring yeast. *Biochimica et biophysica acta* **665**, 596–601.
- BEAVEN, M. J., CHARPENTIER, C. & ROSE, A. H. (1982). Production and tolerance of ethanol in relation to phospholipid fatty-acyl composition in *Saccharomyces cerevisiae* NCYC 431. *Journal of General Microbiology* **128**, 1447–1455.
- BLATIAK, A., GONDAL, J. A. & WISEMAN, A. (1980). Mechanism of degradation of cytochrome P-450 in non-growing *Saccharomyces cerevisiae*: anaerobic, chloramphenicol and cycloheximide as protective agents. *Biochemical Society Transactions* **8**, 711–712.
- BLATIAK, A., KING, D. J., WISEMAN, A., SALIHON, J. & WINKLER, M. A. (1985a). Enzyme induction by oxygen in the accumulation of cytochrome P-450 during batch fermentations in 20% D-glucose with *Saccharomyces cerevisiae*. *Enzyme and Microbial Technology* **7**, 553–556.
- BLATIAK, A., KING, D. J. & WISEMAN, A. (1985b). Effect of alcohols and of glucose on the level of cytochrome P-450 in *Saccharomyces cerevisiae* after resuspension in buffer. *Biochemical Society Transactions* **13**, 924.
- BROWN, S. W., OLIVER, S. G., HARRISON, D. E. F. & RIGHELATO, R. C. (1981). Ethanol inhibition of yeast growth and fermentation: differences in the magnitude and complexity of the effect. *European Journal of Applied Microbiology and Biotechnology* **11**, 151–155.
- CALLEN, D. F., WOLF, C. R. & PHILPOT, R. M. (1980). Cytochrome P-450 mediated genetic activity and cytotoxicity of seven halogenated aliphatic hydrocarbons in *Saccharomyces cerevisiae*. *Mutation Research* **77**, 55–63.
- CASEY, G. P., MAGNUS, C. A. & INGLEDEW, W. M. (1983). High gravity brewing: nutrient enhanced production of high concentrations of ethanol by brewing yeast. *Biotechnology Letters* **5**, 429–434.
- COON, M. J., KOOP, D. R., REEVE, L. E. & CRUMP, B. L. (1984). Alcohol metabolism and toxicity: role of cytochrome P-450. *Fundamental Applied Toxicology* **4**, 134–143.
- DEL CARRATORE, R., MORGANTI, C., GALLI, A. & BRONZETTA, G. (1984). Cytochrome P-450 inducibility by ethanol and 7-ethoxycoumarin O-deethylation in *S. cerevisiae*. *Biochemical and Biophysical Research Communications* **123**, 186–193.
- GUARENTE, L. & MASON, T. (1983). Heme regulates transcription of the CYC1 gene of *S. cerevisiae* via an upstream activation site. *Cell* **32**, 1279–1286.
- GUDENUS, R., SPENCE, A., HARTIG, A., SMITH, M. & RUIS, H. (1984). Regulation of transcription of the *Saccharomyces cerevisiae* CYC1 gene: identification of a DNA region involved in heme control. *Current Genetics* **8**, 45–48.
- GUIJARRO, J. M. & LAGUNAS, R. (1984). *Saccharomyces cerevisiae* does not accumulate ethanol against a concentration gradient. *Journal of Bacteriology* **160**, 874–878.
- HORTNER, H., AMMERER, G., HARTTER, E., HAMILTON, B., RYTKA, J., BILINSKI, T. & RUIS, H. (1982). Regulation of synthesis of catalases and iso-1-cytochrome *c* in *Saccharomyces cerevisiae* by glucose, oxygen and heme. *European Journal of Biochemistry* **128**, 179–184.
- INGELMAN-SUNDBURY, M. & HAGBJORK, A. L. (1982). On the significance of the cytochrome P-450-dependent hydroxyl radical mediated oxygenation mechanism. *Xenobiotica* **12**, 673–686.
- JONES, R. P. & GREENFIELD, P. F. (1985). Replicative inactivation and metabolic inhibition in yeast ethanol fermentations. *Biotechnology Letters* **7**, 223–228.
- KARENLAMPI, S. O., MARTIN, E. & HANNINEN, O. O. P. (1981). Effect of carbon source on the accumulation of cytochrome P-450 in the yeast *Saccharomyces cerevisiae*. *Biochemical Journal* **194**, 407–413.
- KELLY, D. & PARRY, J. M. (1983). Metabolic activation of cytochrome P-450/P-448 in the *Saccharomyces cerevisiae*. *Mutation Research* **108**, 147–159.
- KING, D. J. (1982). *Studies on the biosynthesis and specificity of cytochrome P-450 in the yeast Saccharomyces cerevisiae*. PhD thesis, University of Surrey, UK.
- KING, D. J., AZARI, M. R. & WISEMAN, A. (1982). The induction of cytochrome P-448 dependent benzo(a)pyrene hydroxylase in *Saccharomyces cerevisiae*. *Biochemical and Biophysical Research Communications* **105**, 1115–1121.
- KING, D. J., WISEMAN, A. & WILKIE, D. (1983). Studies on the genetic regulation of cytochrome P-450 production in *Saccharomyces cerevisiae*. *Molecular and General Genetics* **192**, 466–470.
- KING, D. J., AZARI, M. R. & WISEMAN, A. (1984). Studies on the properties of highly purified cytochrome P-448 and its dependent activity benzo(a)pyrene hydroxylase from *Saccharomyces cerevisiae*. *Xenobiotica* **14**, 187–206.
- LAZ, T. M., PIETRAS, D. F. & SHERMAN, F. (1984). Differential regulation of the duplicated isocytochrome *c* genes in yeast. *Proceedings of the National Academy of Sciences of the United States of America* **81**, 4475–4479.
- LEAO, C. & VAN UDEN, N. (1982a). Effects of ethanol and other alkanols on the kinetics and the activation

- parameters of thermal death in *Saccharomyces cerevisiae*. *Biotechnology and Bioengineering* **24**, 2601–2604.
- LEAO, C. & VAN UDEN, N. (1982*b*). Effects of ethanol and other alkanols on the glucose transport system of *Saccharomyces cerevisiae*. *Biotechnology and Bioengineering* **24**, 2601–2604.
- LEAO, C. & VAN UDEN, N. (1983). Effects of ethanol and other alkanols on the ammonium transport system of *Saccharomyces cerevisiae*. *Biotechnology and Bioengineering* **25**, 2085–2090.
- LEAO, C. & VAN UDEN, N. (1984*a*). Effects of ethanol and other alkanols on the general amino acid permease of *Saccharomyces cerevisiae*. *Biotechnology and Bioengineering* **26**, 403–405.
- LEAO, C. & VAN UDEN, N. (1984*b*). Effects of ethanol and other alkanols on passive proton influx in the yeast *Saccharomyces cerevisiae*. *Biochimica et biophysica acta* **774**, 43–48.
- LEAO, C. & VAN UDEN, N. (1985). Effects of ethanol and other alkanols on the temperature relations of glucose transport and fermentation in *Saccharomyces cerevisiae*. *Applied Microbiology and Biotechnology* **22**, 359–363.
- LOUREIRO, V. & FERREIRA, H. G. (1983). On the intracellular accumulation of ethanol in yeast. *Biotechnology and Bioengineering* **25**, 2263–2269.
- MORITA, T. & MIFUCHI, I. (1984). Ethanol enhancement of cytochrome P-450 in yeast *Saccharomyces cerevisiae* D7. *Chemical Pharmacology Bulletin* **32**, 1624–1627.
- MULLER, R., SCHMIDT, W. E. & STIER, A. (1985). The site of cyclic AMP-dependent protein kinase catalysed phosphorylation of cytochrome P-450 LM2. *FEBS Letters* **187**, 21–24.
- ONURA, T. & SATO, R. (1964). The carbon monoxide binding pigment of liver microsomes. I. Evidence for its haemoprotein in nature. *Journal of Biological Chemistry* **239**, 2370–2379.
- PROGNON, P., BLAIS, J., VIGNY, P., AVERBECK, D., AVERBECK, S. & GOND, A. (1984). The metabolism of 8-methoxypsoralen by *Saccharomyces cerevisiae*. Evidence for an inducing effect of ethanol. *II Farmaco Edizione Scientifica* **39**, 739–751.
- ROSS, E. & SCHATZ, G. (1976). Cytochrome c_1 of Baker's yeast. II. Synthesis on cytoplasmic ribosomes and influences of oxygen and heme on accumulation of the apoprotein. *Journal of Biological Chemistry* **251**, 1997–2004.
- SAUER, M., KAPPELI, O. & FIECHTER, A. (1982). Comparison of the cytochrome P-450 containing monooxygenases originating from two different yeasts. In *Cytochrome P-450: Biochemistry, Biophysics and Environmental Implications*, pp. 453–457. Edited by E. Hietanen, M. Laitinen & O. Hanninen. Amsterdam: Elsevier Biomedical Press.
- THOMAS, D. S., HOSSACK, J. A. & ROSE, A. H. (1978). Plasma membrane lipid composition and ethanol tolerance in *Saccharomyces cerevisiae*. *Archives of Microbiology* **117**, 239–245.
- WALKER-CAPRIOLLO, H. M., RODRIGUES, R. J. & PARKS, L. W. (1985). Recovery of *Saccharomyces cerevisiae* from ethanol-induced growth inhibition. *Applied and Environmental Microbiology* **50**, 685–689.
- WISEMAN, A. & WOODS, L. F. J. (1979). Benzo(a)pyrene metabolites formed by the action of yeast cytochrome P-450/P-448. *Journal of Chemical Technology and Biotechnology* **29**, 320–324.
- WISEMAN, A., LIM, T. K. & MCCLOUD, C. (1975). Relationship of cytochrome P-450 to growth phase of Brewer's yeast in 1% or 20% glucose medium. *Biochemical Society Transactions* **3**, 276–278.
- WISEMAN, A., LIM, T. K. & WOODS, L. F. J. (1978). Regulation of the biosynthesis of cytochrome P-450 in Brewer's yeast. Role of cyclic AMP. *Biochimica et biophysica acta* **544**, 615–623.