Hydroxylation of Alkanes and Fatty Acids in *Saccharomycopsis lipolytica*. Evidence for the Involvement of Cytochrome P-450

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Growth of the yeast *Saccharomycopsis lipolytica* on *n*-alkanes induced a hydroxylation system which, in the presence of NADPH and O₂, converted lauric acid into ω -hydroxylauric acid. The specific hydroxylation activity was lower with the physiological substrate *n*-hexadecane than with lauric acid. This system was particulate and did not act on *N*- and *O*-dealkylated compounds. It was strongly inhibited by CO and its enzymic activity was unstable. Because of the presence of cytochrome oxidase in the preparations, spectral demonstration of the presence of cytochrome P-450 was difficult but could be achieved with the techniques developed.

INTRODUCTION

The first step in the degradation of *n*-alkanes in yeasts and other micro-organisms is the hydroxylation of the terminal methyl group (Ratledge, 1978). In *Candida tropicalis* (Lebeault *et al.*, 1971; Gallo *et al.*, 1971) this reaction is catalysed by a monooxygenase which requires O₂ and NADPH, NADH having an effect synergistic with that of NADPH. The hydroxylation system of *C. tropicalis* has been partially purified and resolved into three components: a cytochrome P-450, a cytochrome P-450 reductase and a thermostable lipid (Duppel *et al.*, 1973). This, as well as the capacity to demethylate several drugs (Schunck *et al.*, 1978*a*), suggests a marked similarity between the yeast and the mammalian liver microsomal system.

A microsomal system also involving cytochrome P-450 has been reported in *Candida guilliermondii*. This system converts *n*-hexadecane into *n*-hexadecanol and palmitic acid (Schunck *et al.*, 1978*b*). As in the case of *C. tropicalis*, cytochrome P-450 is induced by growth on *n*-alkanes (Tittelbach *et al.*, 1976).

Among alkane-utilizing yeasts, *Saccharomycopsis lipolytica* stands out as the organism which has been the most thoroughly studied at the biochemical and genetic levels. However, in this yeast, the nature of the system of initial degradation of *n*-alkanes has not been clearly established. According to Delaissé & Nyns (1974), the hydroxylating system also consists of a cytochrome P-450. However, Peterson (1970) did not succeed in showing the presence of cytochrome P-450 in cell-free extracts of this yeast. Moreover, Rohde *et al.* (1975) briefly reported that oxidation of *n*-hexadecane by extracts of *S. lipolytica* required NADH but not NADPH.

This present work was undertaken in order to obtain a better understanding of the *n*-alkane hydroxylation system of *S. lipolytica*, and the properties of this system are described below.

METHODS

Organisms and culture. *Saccharomycopsis lipolytica* 102 was grown in batch culture in a stirred fermenter as previously described (Marchal *et al.*, 1977), except that *n*-hexadecane replaced the *n*-paraffin cut as the carbon source and NH_4OH replaced KOH as the pH-regulating agent. After 20 h of growth, the cells were harvested by centrifugation (48 000 *g* for 10 min at 4 °C). The yeast pellet was immediately washed with 50 mM-potassium phosphate buffer (pH 7.0) to remove residual *n*-hexadecane. *Candida tropicalis* CBS 6947 was grown and harvested in exactly the same manner.

Enzyme preparations. A suspension of 8 g (wet wt) organisms in 8 ml 50 mM-potassium phosphate buffer (pH 7.0) was shaken with 16 g glass beads (0.5 mm diam.) in a refrigerated ball mill (Braun MSK) for 1.5 min. The broken organisms were centrifuged in two steps, first for 5 min at 1000 *g* and then for 15 min at 10 000 *g* in order to prevent the smaller particles being carried along in the pellet. The supernatant, which constituted the crude extract, was centrifuged for 90 min at 100 000 *g* in order to recover the particulate fraction. A suspension of this pellet in the same phosphate buffer could be kept frozen at -80 °C for several months without any significant loss of hydroxylation activity. The rat liver extract was a microsomal preparation obtained from rats induced by Aroclor 1254 (Ames *et al.*, 1975).

Determination of the hydroxylation activity with hexadecane and laurate. The reaction was performed under air at 30 °C in 25 ml sealed glass vials which were agitated at 200 rev. min⁻¹. The incubation time was 20 min (unless otherwise stated). The reaction mixture (1 ml) had the following composition (unless otherwise stated): 50 µmol potassium phosphate buffer (pH 7.0), 2.5 µmol NADPH, 2.5 µmol NADH, 20 µmol sodium isocitrate, 38 µmol glucose, NADP⁺-linked isocitrate dehydrogenase (EC 1.1.1.42) from pig heart (20 U), glucose dehydrogenase (EC 1.1.1.47) from *Bacillus subtilis* (1.5 U), 124 nmol [¹⁻¹⁴C]laurate or *n*-[1-¹⁴C]hexadecane (833 Bq) dissolved in 350 µmol ethanol, and cell-free extract (1–4 mg protein). In the case of laurate, which is water-soluble at the concentration used, the reaction was initiated by addition of the laurate solution in ethanol. In the case of hexadecane, which is practically insoluble in water, emulsification of the reaction mixture containing all components except NADPH was performed by sonication (15 s at 0 °C) before initiation of the reaction by addition of NADPH.

The reaction was stopped by the addition of 50 µl 50% (v/v) H_2SO_4 . The acidified medium was agitated for 1 min with 4 ml ether. The two phases were separated by centrifugation (5 min at 10 000 *g*) and the organic phase was easily withdrawn after freezing the aqueous phase in a bath of acetone/solid CO_2 . After evaporation of the ether phase in a 5 ml vial ('reacti-vial' from Pierce, Rockford, Ill., U.S.A.), the residue was taken up twice with two 0.1 ml amounts of ether and applied to a thin-layer plate of silica gel. The plate was developed with ether/hexane/acetic acid (60:40:1, by vol.). The composition of this mixture had been selected for optimal separation between lauric acid and its oxidation products. The positions of the radioactive spots were detected by autoradiography (exposure time of 3 d at 4 °C). The spots were scraped off and placed in vials containing 15 ml of a scintillator solution [4 g 2,5-diphenyloxazole and 0.5 g *p*-(*O*-methylstyryl)benzene per litre toluene]. The radioactivity of the residual substrate was used as an internal standard for the calculation of enzymic activity which included all products.

In preliminary experiments, we compared the separation obtained with silicic acid columns (Kusunose *et al.*, 1964) and with thin-layer plates. The second method gave the best results. We have also compared the separation obtained on thin-layer plates with or without preliminary ether extraction. The direct application of the sample was retained only in the case of assays with *n*-hexadecane as the substrate in the presence of detergent, the extraction with ether being ineffective in this case.

Determination of the hydroxylation activity with *O*- and *N*-alkylated compounds. Oxidations of *O*- and *N*-alkylated compounds were performed as described by Duppel *et al.* (1973) with aminopyrine, anisole, dimethylnaphthylamine or methylamine as substrates, using the reagent described by Nash (1953) to measure the formaldehyde produced by the demethylation reaction. Demethylation of [dimethylamine-¹⁴C]aminopyrine was also estimated using the method of Poland & Nebert (1972).

Dealkylation of ethoxycoumarin was measured by a modification of the fluorimetric method of Ullrich & Weber (1972). The apparatus used was an Eppendorf photometer equipped with the fluorimetric kit 1030 (passing bands: 313–360 nm for primary filter and 470–3000 nm for secondary filter). In our assay, in order to minimize interference due to the fluorescence of NADPH and NADH, the initial concentration of these compounds was lowered to 16 µM for NADPH and 10 µM for NADH and the concentrations were kept constant during the course of the reaction by use of the reduced pyridine nucleotide-regenerating systems mentioned above. The minimum activity detectable was 5 pmol min⁻¹ (mg protein)⁻¹.

Spectrophotometric examination. Examinations of the CO-complexes of cytochromes were carried out on particulate fractions with a Beckman 25 spectrophotometer. The reduced *minus* reduced + CO difference spectra (Omura & Sato, 1964) and the reduced + CO *minus* oxidized + CO spectra (Schoene *et al.*, 1972) were investigated.

Protein determination. The protein content of the extracts was measured by the method of Lowry.

Materials. *n*-[1-¹⁴C]Hexadecane, [1-¹⁴C]lauric acid and [dimethylamine-¹⁴C]aminopyrine were purchased from Amersham and [1-¹⁴C]myristic acid, [1-¹⁴C]palmitic acid and [1-¹⁴C]stearic acid from the Commissariat à l'Energie Atomique, Gif sur Yvette, France. ω -Hydroxylauric acid was obtained from Aldrich Europe, Beerse, Belgium. Glucose dehydrogenase and NADP⁺-linked isocitrate dehydrogenase were from Boehringer. The thin-layer chromatography plates of silica gel 60 (20 × 20 cm, layer thickness 0.25 mm) were from Merck. Autoradiography was carried out using a Kodirex radiographic film (Kodak). *n*-[1-¹⁴C]Hexadecane, which was contaminated with about 1% of an impurity identified as *n*-hexadecanol, was purified by thin-layer chromatography before use.

RESULTS

Characterization of the hydroxylation system

Cofactor requirements. Initial experiments were carried out with laurate as the substrate. Figure 1 shows an autoradiogram of the thin-layer chromatographic separation performed on the products formed in assays carried out under various conditions. Laurate was oxidized to ω -hydroxylaurate under the conditions of assay 1. In assay 5, ω -hydroxylaurate was again produced but a further oxidation to the dicarboxylic acid also occurred. Both products were identified by comparison of their R_F values with those of the pure reference compounds. In addition, the formation of dodecanedioate confirmed that the initial hydroxylation of laurate took place on the terminal methyl group. The hydroxylation activities presented in the legend of Fig. 1 show that oxidation of laurate by a particulate extract of *S. lipolytica* requires the presence of both O₂ and NADPH. A much lower, but nevertheless significant activity, was found when NADPH was replaced by NADH.

The synergistic effect of NADPH and NADH on hydroxylation activity, demonstrated in Table 1, suggests that the role of NADH was not merely to allow the functioning of an independent NADH-linked hydroxylation system. Table 1 also shows that the hydroxylation activity was increased in the presence of regenerating systems of NADH and NADPH; while Table 2 shows that this increase could be ascribed to the regeneration of NADH, but not of NADPH. This effect is probably related to the fact that, in crude extracts, the NADH oxidase activity [28 nmol min⁻¹ (mg protein)⁻¹] was appreciably higher than the corresponding NADPH oxidase activity [5 nmol min⁻¹ (mg protein)⁻¹]. The replacement of NADPH and O₂ by hydrogen peroxide or by cumene hydroperoxide both at a concentration of 3.3 mM (Nordblom *et al.*, 1976) was unsuccessful.

Substrate specificity. The specificity of the enzymic system is illustrated in Tables 3 and 4. All the fatty acids tested and, in particular, laurate were well utilized (Table 3). On the contrary, with the physiological substrate, *n*-hexadecane, the activity was surprisingly low (about 1% of the activity with laurate). The addition of Triton X-100 or cholate at 1 g l⁻¹ or Tween 80 at 1, 5, 10 or 20 g l⁻¹ did not markedly improve the activity with this substrate. Control experiments (Table 4) were performed with rat liver microsomes, which are known to hydroxylate alkanes (Das *et al.*, 1968), and with extracts of *C. tropicalis* CBS 6947; activities of the same order of magnitude were obtained. These results are clearly different from those of Lebeault *et al.* (1971), who reported a hydroxylation activity as high as 800 pmol min⁻¹ (mg protein)⁻¹ in *C. tropicalis* LM 7 extracts but only four times more activity for laurate.

Methylamine, dimethylnaphthylamine and anisole were not dealkylated with significant production of formaldehyde measured as described in Methods.

General properties of the hydroxylation system. Phosphate buffer was the best of the various buffers tested (Table 5, expt 1) and the activity in this buffer was found to be maximum at pH 7.0 (Table 5, expt 2). With phosphate buffer, the velocity of the reaction with 124 μ M-laurate and a crude extract (2.5 mg protein ml⁻¹) was approximately constant for 15 min and slowly declined up to 25 min. The velocity of the reaction with 124 μ M-laurate was also proportional to the amount of crude extract used up to 3.5 mg protein ml⁻¹.

Fig. 1. Characterization of the hydroxylation activity for laurate and of its products in *S. lipolytica* cell-free preparations, by autoradiography of the chromatogram obtained for assays performed in the following conditions. Assay mixtures contained a particulate fraction ($1.33 \text{ mg protein ml}^{-1}$, except in assay 5 which contained $6.65 \text{ mg protein ml}^{-1}$), $124 \mu\text{M}$ -laurate, 50 mM -potassium phosphate ($\text{pH } 7.5$) and the coenzymes indicated below, each used at a concentration of 2.5 mM , but no reduced pyridine nucleotide regenerating systems. The incubation period was 15 min (except in assay 5, where it was 30 min). The coenzymes used in each assay and the corresponding hydroxylation activities [$\mu\text{mol min}^{-1} (\text{mg protein})^{-1}$] obtained were: assay 1 – NADPH, 315; 2 – NADH, 50; 3 – NAD $^{+}$ and NADP $^{+}$, 10; 4 – no coenzyme, 33; 5 – NADPH and NADH, 193; 6 – NADPH, 38. All the assays were performed aerobically (except assay 6 which was incubated under N_2). The particular conditions of assay 5 (large amount of extract, longer period of incubation) were chosen to maximize the formation of products for identification purposes; as a consequence, the enzymic activity measured in this assay was no longer linear versus time and protein concentration. Products: A, ω -hydroxylaurate; B, dodecanedioate; C, laurate; D, unidentified product.

Table 1. Synergistic effects of NADPH and NADH in laurate hydroxylation

A particulate fraction from *S. lipolytica* was used. The substrate was $124 \mu\text{M}$ -laurate, and assay mixtures contained the coenzymes indicated (each used a concentration of 2.5 mM). Reduced pyridine nucleotide regenerating systems were added only where indicated.

Coenzyme(s)	Hydroxylation activity [$\mu\text{mol min}^{-1} (\text{mg protein})^{-1}$]
NADPH	332
NADH	120
NADPH + NADH	638
NADPH + NADH	1376*

* NADPH and NADH regenerating systems added.

Table 6 shows that the hydroxylation activity was localized in the particulate fraction. The instability of the activity of the system (see below) probably explains the lower activity found in the reconstituted extract.

The hydroxylation system was induced by growth of the yeast on *n*-hexadecane, as in *C. tropicalis* (Gilewicz *et al.*, 1979). The activity with laurate in particulate preparations from

Table 2. *Effect of the reduced pyridine nucleotide regenerating systems on the hydroxylation activity*

A crude extract of *S. lipolytica* was used (2.5 mg protein ml⁻¹ in the assay mixture). The substrate was 124 µM-laurate, and assay mixtures contained the coenzymes (each used at a concentration 2.5 mM) and reduced pyridine nucleotide generating systems indicated. The incubations were performed in 50 µM-potassium phosphate buffer (pH 7.5).

Coenzyme(s)	Reduced pyridine nucleotide generating system(s)	Hydroxylation activity [pmol min ⁻¹ (mg protein) ⁻¹]
None	None	1
NADPH	None	64
NADPH	NADPH	66
NADH	None	11
NADH	NADH	42
NADPH + NADH	NADPH + NADH	180

Table 3. *Hydroxylation activities with fatty acid substrates*

A crude extract from *S. lipolytica* was used. The initial concentration of each substrate was 124 µM.

Substrate	Hydroxylation activity [pmol min ⁻¹ (mg protein) ⁻¹]
Laurate (C ₁₂)	812
Myristate (C ₁₄)	116
Palmitate (C ₁₆)	81
Stearate (C ₁₈)	150

Table 4. *Hydroxylation activities for n-hexadecane and laurate of S. lipolytica, C. tropicalis and rat liver*

Crude extracts were used except in the case of *C. tropicalis* CBS 6947 where a particulate fraction was utilized. The substances were each at an initial concentration of 124 µM.

Origin of extract	Substrate	Hydroxylation activity [pmol min ⁻¹ (mg protein) ⁻¹]
<i>S. lipolytica</i>	<i>n</i> -Hexadecane (control, 0 min)	0.8
	<i>n</i> -Hexadecane	6.7
	Laurate	568
<i>C. tropicalis</i>	<i>n</i> -Hexadecane	8.0
	Laurate	1720
Rat liver	<i>n</i> -Hexadecane	3.4
	Laurate	155

glucose-grown cultures [92 pmol min⁻¹ (mg protein)⁻¹] was, in fact, several times lower than that of corresponding preparations obtained from cultures grown on *n*-hexadecane [722 pmol min⁻¹ (mg protein)⁻¹]. Lebeault *et al.* (1971) also reported a residual activity in cultures of *C. tropicalis* grown on glucose.

Hydroxylation activity was sensitive to inhibition by CO: when air in the assay vial was replaced by O₂/CO (4 : 1, v/v), the activity was reduced by 86% [from 301 to 43 pmol min⁻¹ (mg protein)⁻¹].

The stability of the hydroxylation system of *S. lipolytica* was found to be lower than that of *C. tropicalis*. Crude extracts stored at 4 °C lost 78% activity in 24 h in the case of *S. lipolytica* 102 and only 5% activity in the case of *C. tropicalis* CBS 6947. The addition of 30% glycerol, 1 mM-dithiothreitol, 1 mM-EDTA or 4 mM-phenylmethylsulphonyl fluoride or

Table 5. *Effect of the nature of the buffer and pH on the hydroxylation activity*

Crude extracts were used. The substrate was 124 μ M-laurate. The incubations were performed in 50 mM-buffer at the indicated pH. ω -Hydroxylaurate represented 95–97% and dodecanedioate 3–5% of the products formed.

Expt no.	Buffer*	pH	Hydroxylation activity [pmol min ⁻¹ (mg protein) ⁻¹]
1	Potassium phosphate	7.0	670
	Tris/HCl	7.4	240
	Imidazole	7.0	40
	HEPES	7.0	260
	MOPS	7.0	160
	TES	7.0	260
2	Potassium phosphate	6.4	550
	Potassium phosphate	6.8	840
	Potassium phosphate	7.0	920
	Potassium phosphate	7.2	840
	Potassium phosphate	7.4	540
	Potassium phosphate	7.7	370

* HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonate; MOPS, 3-(*N*-morpholino)propanesulphonate; TES, *N*-tris(hydroxymethyl)methyl-2-aminoethanesulphonate.

Table 6. *Localization of hydroxylation activity in S. lipolytica*

The substrate was 124 μ M-laurate. The particulate fraction was obtained by ultracentrifugation of the crude extract as described in Methods. After resuspension in 50 mM-potassium phosphate buffer (pH 7.0), it was recentrifuged once for washing.

Incubation condition	Hydroxylation activity [pmol min ⁻¹ (mg protein) ⁻¹]
Control (crude extract, 0 min incubation)	1
Crude extract	357
Particulate fraction	435
Soluble fraction	17
Reconstituted crude extract (soluble plus particulate fractions)	161

storage under N₂ did not improve the stability of the *S. lipolytica* system. The activity in the presence of glycerol, however, was about twice that measured in its absence.

Spectral examination. In order to detect the presence of cytochrome P-450, particulate preparations of *S. lipolytica* incubated with CO were examined under the conditions of Omura & Sato (1964). For comparison, similar experiments were carried out with *C. tropicalis* CBS 6947 and rat liver microsomal preparations. The spectra obtained (Fig. 2) clearly show in the microsomes of rat liver (spectrum 1*a*) and in *C. tropicalis* (spectrum 1*b*) the presence of the characteristic peak at 450 nm of cytochrome P-450. In the case of *S. lipolytica* (spectrum 1*c*), there was no evidence for the presence of this cytochrome in the particulate fraction. The spectrum presented a peak at about 427 nm and a trough at 443 nm, which completely masked any cytochrome P-450 present in the preparation. In fact, the characteristics of this spectrum indicated the presence in our preparation of cytochrome *c* oxidase (Okunuki, 1966). The trough at 443 nm resulted from the absorption of the Soret band of the reduced form of cytochrome oxidase. The procedure used by Schoene *et al.* (1972) to circumvent the interference of haemoglobin could be used here, since the Soret absorption peaks of the reduced + CO and oxidized forms of cytochrome oxidase both occur

Fig. 2. Spectral detection of cytochrome P-450 in particulate preparations. (1) Differential spectra according to Omura & Sato (1964) (reduced + CO *minus* reduced) and (2) differential spectra according to Schoene *et al.* (1972) (reduced + CO *minus* non-reduced + CO): (a) rat liver; (b) *C. tropicalis* CBS 6947; (c) *S. lipolytica*.

The shoulder at 426 nm in spectrum 2a can be attributed to the presence of cytochrome b_5 in the rat liver preparation (Estabrook & Werringloer, 1978) which may also contribute to spectra 2b and 2c. Spectra quite similar to spectra 1b and 2b have been observed for another alkane-grown strain, *C. tropicalis* I.F.P. 10.

in the range 420–430 nm (Vanneste, 1966). The spectra obtained with this procedure indicated the presence of cytochrome P-450 in *S. lipolytica* extracts (spectrum 2c). However, the concentration was lower than in the preparations of rat liver (spectrum 2a) and *C. tropicalis* (spectrum 2b).

DISCUSSION

The results presented here clearly establish that, as in *C. tropicalis* (Lebeault *et al.*, 1971; Gallo *et al.*, 1971; Duppel *et al.*, 1973), the first step in the degradation of *n*-alkanes in *S. lipolytica* involves a monooxygenase system which requires O_2 and NADPH, NADH having an effect synergistic with that of NADPH. As usually observed for hydroxylating systems from alkane-grown yeasts, this enzymic activity was induced by growth on *n*-alkanes but significant activity was present in glucose-grown organisms. Like most other comparable systems from eukaryotic micro-organisms, including the *C. tropicalis* system as described by

Gallo *et al.* (1976) but not by Lebeault *et al.* (1971), the hydroxylase was localized in the particulate fraction. Because of its very low solubility in water, the physiological substrate, *n*-hexadecane, was not rapidly hydroxylated. Although this point has not been stressed by other authors, it probably has some generality as we have also observed it with *C. tropicalis* (Table 4) and *Pseudomonas aeruginosa* (Blanchet & Vandecasteele, unpublished work). As addition of various detergents did not appreciably increase the rate of hexadecane hydroxylation, the question of the manner by which this reaction occurs inside the cell remains unanswered. More soluble compounds such as fatty acids, however, in particular lauric acid, were rapidly transformed into the corresponding ω -hydroxyacids. CO strongly inhibited our hydroxylation system. The *S. lipolytica* system presents many similarities with that described for *Torulopsis magnoliae* (Heinz *et al.*, 1970) which may contain cytochrome P-450 (Wiseman, 1977). In *S. lipolytica*, differential spectrophotometric spectra obtained using the method of Schoene *et al.* (1972) showed the presence of cytochrome P-450. The stability of the system, however, was quite poor and could not be improved by the addition of glycerol or dithiothreitol and EDTA. Because of the presence of cytochrome oxidase which interfered with the spectral measurements, it was not possible to determine from these measurements if the instability of the *S. lipolytica* hydroxylation system was due to denaturation of cytochrome P-450 to P-420, but it is possible that P-420 contributes to spectrum 1c of Fig. 2. Instability prevented the purification required for further characterization of the hydroxylation system, such as recently described in the cases of *C. tropicalis* (Bertrand *et al.*, 1979*a, b*) and *Saccharomyces cerevisiae* (Yoshida *et al.*, 1977). An important difference between our system and that of *C. tropicalis* described by Duppel *et al.* (1973) was that our preparations did not dealkylate *N*- and *O*-alkylated compounds. In fact, the narrow specificity displayed by *S. lipolytica* cytochrome P-450 is well in accord with its physiological role of oxidizing the long-chain alkanes used as carbon sources, just as the wide specificity of the homologous enzymes of mammalian liver reflects their involvement in the detoxification of a large spectrum of xenobiotic compounds.

In hydroxylation systems involving cytochrome P-450, an NADPH-cytochrome *c* reductase can often be characterized as an individual enzyme after solubilization of the system, as recently illustrated in the case of *C. tropicalis* (Bertrand *et al.*, 1979*a, b*) and *Saccharomyces cerevisiae* (Aoyama *et al.*, 1978). Such an activity could not be characterized here because of the presence of an endogenous cytochrome *c* reductase activity in our preparations.

Some of the enzymes allowing further metabolism of alcohols resulting from the action of the hydroxylating system studied have been detected in our preparations. As in *C. tropicalis* (Lebeault *et al.*, 1970), an NAD⁺-dependent alcohol dehydrogenase activity was observed in *S. lipolytica* but it was found only in the soluble fraction of the extract. This activity measured at pH 7.0 with 2 mM-octanol was 12 nmol min⁻¹ (mg protein)⁻¹. Other results (C. Célier & J. P. Vandecasteele, unpublished observations) indicated this activity was about 2.5 times higher in alkane-grown cells than in glucose-grown cells. The pyridine nucleotide-independent alcohol dehydrogenase which has been shown to be specific for alkane oxidation in Gram-negative bacteria (Tassin *et al.*, 1973; Tauchert *et al.*, 1975; Benson & Shapiro, 1976) could not be detected in our preparations. NAD⁺-dependent aldehyde dehydrogenase activity [specific activity at pH 7.0 with 2 mM-octanol was 10 nmol min⁻¹ (mg protein)⁻¹, in crude extracts] was also detected both in the soluble and the particulate fractions of *S. lipolytica* extracts. These activities probably explain the formation of dodecanedioate from ω -hydroxylaurate in our preparations and their postulated role is the oxidation of primary alcohols to fatty acids in alkane degradation.

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