Production, Identification, and Toxicity of γ -Decalactone and 4-Hydroxydecanoic Acid from *Sporidiobolus* spp.

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Received 14 November 1995/Accepted 16 May 1996

During the bioconversion of ricinoleic acid to γ -decalactone under controlled pH conditions, *Sporidiobolus salmonicolor* produced only the lactone form, while *Sporidiobolus ruinenii* produced both the lactone form and a precursor. By using gas chromatography-mass spectrometry and gas chromatography-Fourier transform infrared analysis techniques, the precursor was identified as 4-hydroxydecanoic acid. The levels of production in the presence of high concentrations of ricinoleic acid methyl ester differed in the two *Sporidiobolus* species. This difference was due on the one hand to the high sensitivity of *S. salmonicolor* to the lactone and on the other hand to the high level of 4-hydroxydecanoic acid produced by *S. ruinenii*. 4-Hydroxydecanoic acid is much less toxic to the microorganisms than the lactone. In contrast to *S. ruinenii*, *S. salmonicolor* is not able to catabolize 4-hydroxydecanoic acid.

It has been known since 1930 that γ -decalactone is produced by members of the genus *Sporidiobolus*, and this fact was confirmed by the identification of 4-decanolide and *cis*-6-dodecanolide in 1972 and 1973 (31, 32) in the peach-like odor of *Sporobolomyces odorosus*. After this, many lactone-producing microorganisms were identified (1–3, 11, 27). Lactones are widely distributed in food and beverages (9) and have been found in the aromas of more than 120 foodstuffs, which explains their importance to the aroma industry. Because of the increasing demand for natural products, biotechnological processes for the production of lactones have been developed. Generally, these processes involve the bioconversion of a hydroxy fatty acid to a γ - or δ -lactone (4, 10, 13, 15, 29). This has yielded numerous patents. Today, γ -decalactone is the principal biotechnologically derived product of the aroma industry, but some γ -dodecalactone and some γ -octalactone are also produced. From a metabolic point of view, the pathway involved in the catabolism of ricinoleic acid to γ -decalactone was described for *Candida* spp. by Okui et al. (25) in 1963. Among other things, these authors concluded that the lactone seemed to be derived from 4-hydroxydecanoic acid, but whether lactonization occurred in vivo or took place during isolation was not determined. More recently, in patents and papers on the subject (10, 19, 29) workers have described acidification and heating of the culture medium as necessary steps for full recovery of the lactone (6, 7, 29). This implies the presence of a precursor of γ -decalactone in the culture broth. The precursor is thought to be the corresponding free hydroxy fatty acid, but no data on either its isolation or its spectral characteristics have been published. However, as the treatments for maximizing recovery of lactone are usually used routinely, it is impossible to know if the acid is always formed during the production of γ -decalactone from ricinoleic acid regardless of the micro-

organism used. The place of the free acid in the metabolic pathway remains unknown.

In this paper, we describe the production and identification of γ -decalactone and 4-hydroxydecanoic acid by two *Sporidiobolus* species. Spectral data for the acid are given for the first time. The effects of the two compounds on the viability of the microorganisms were also studied. The metabolic events leading to the synthesis of the two molecules are discussed below.

MATERIALS AND METHODS

Strains, growth media, and culture conditions. *Sporidiobolus salmonicolor* CBS 2636 and *Sporidiobolus ruinenii* CBS 5001 cultures were grown in 7-liter fermentors containing 5 liters of medium consisting of 15 g of glucose per liter, 0.5 g of Bacto Tryptone (Difco) per liter, 1 g of yeast extract per liter, 1 g of malt extract per liter, 2 g of Casamino Acids per liter, 2 g of KH_2PO_4 per liter, 0.13 g of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ per liter, 0.01 g of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ per liter, 3 g of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ per liter, and 0.1% (vol/vol) antifoam. The medium was sterilized at 121°C for 20 min. Inoculation (2%, vol/vol) was performed with a yeast suspension (6×10^8 cells per ml) that had been cultivated previously in the same medium. The cultures were stirred (250 rpm) and were aerated (1 volume of air per volume of medium per minute) with a volumetric oxygen transfer coefficient of 90 h^{-1} , calculated as described by Rainer (26). The pH was kept at 6.0 by adding 2.5 N NaOH or 2.5 N H_2SO_4 . Methyl ricinoleate (5%, vol/vol) was added to the medium after 24 h of culture to initiate the bioconversion process.

Viability. A 1-ml portion of a culture was diluted 10^6 -fold with a sterile 0.85% NaCl solution. After vortex mixing, 100- μl portions of the dilution were plated onto petri dishes filled with the medium described above supplemented with agar (20 g/liter). Colonies were counted after 48 h of incubation at 25°C for *Sporidiobolus ruinenii* and after 96 h of incubation for *Sporidiobolus salmonicolor*.

Effects of γ -decalactone and 4-hydroxydecanoic acid on growth kinetics. Cultures were grown in 500-ml flasks containing 100 ml of the medium described above supplemented with γ -decalactone and 4-hydroxydecanoic acid at concentrations ranging from 100 to 500 mg/liter. The flasks were inoculated as described above and were incubated at 24°C with shaking at 180 rpm on a rotary shaker.

Growth was evaluated by measuring the apparent optical density at 620 nm of 100 μl of culture diluted to a volume of 4 ml with distilled water. Growth curves were fitted by using the logistic fit program developed by SigmaPlot and the following equation: function $\text{OD} = (a - d)/[1 + (t/c)^b]^{1/e} + d$, where OD is the optical density, a and d are the asymptotic minimum and maximum values, b is the slope parameter, c is the t value at the asymptotic point, and e is a symmetry parameter. After curve fitting, the specific growth rate (μ) was calculated as described by Jason (17), as follows: $\mu = \Delta\text{OD}/(\text{OD} \cdot \Delta t)$, where ΔOD is the difference between the two optical density values used, OD is the mean of the two optical density values used, and Δt is the interval time.

Aroma analysis. (i) Extraction procedure: fermentor experiments. Aroma extractions were performed by the method of Likens and Nickerson (21) with 30 ml of twice-distilled CH_2Cl_2 per 100 ml of culture medium supplemented with 100 mg of γ -undecalactone as an internal standard. In some cases, the culture pH was adjusted to 2.0 prior to extraction. To study the distribution of the lactone, 100 ml of a culture was centrifuged at $2,000 \times g$ for 5 min to remove the cells and

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to separate the aqueous and oil phases. The volume of each fraction was determined, and each fraction was diluted to a volume of 100 ml with distilled water prior to extraction.

Intermediates of ricinoleic acid metabolism were isolated as follows. Calcium chloride was added to 100 ml of an aqueous phase (obtained as described above) until a precipitate was formed. The precipitate was recovered after 5 min of centrifugation at $2,000 \times g$. The resulting crystals were washed three times with double-distilled water, dissolved in 200 mM phosphate buffer (pH 8.0), and dried under a vacuum. The resulting crystals were esterified with 1 ml of a freshly distilled ethereal diazomethane solution. The resulting methyl esters were dried under nitrogen and resuspended in 1 ml of distilled ether for use in gas chromatography-mass spectrometry, positive chemical ionization, and gas chromatography-Fourier transform infrared analyses.

(ii) **Extraction procedure: toxicity experiments.** γ -Decalactone and 4-hydroxydecanoic acid concentrations were estimated every 8 h. A 1-ml portion of medium acidified to pH 2.0 with HCl was extracted three times with 0.5 ml of diethyl ether containing 0.5 g of γ -undecalactone per liter as an internal standard. The extracts were analyzed as described below.

(a) **Gas chromatography.** Volatile compounds were analyzed with a type DB-FFAP bonded fused silica capillary column (30 m by 0.32 mm [inside diameter]; film thickness, 0.25 μ m; J & W Scientific, Inc.). Injection (1 μ l) was splitless/split (30 s). The temperature was increased from 40 to 240°C at a rate of 3°C/min. The hydrogen carrier gas velocity was 50 cm/s at room temperature. The lactone gas chromatographic peaks were integrated with a multichannel chromatography Coconut workstation (R. Almanza and P. Mielle, Institut National de la Recherche Agronomique, Dijon, France).

(b) **Mass spectrometry and infrared spectroscopy.** Mass spectra were obtained with a Nermag model R10-10 apparatus coupled to a model HP5890 gas chromatograph (Hewlett-Packard). The column and the gas chromatography conditions were the same as those described above except that helium (velocity, 35 cm/s) was used as the carrier gas. Electron impact spectra were recorded with an ion source energy of 70 eV. The scanning rate was 1 s from 25 to 300 atom mass units. Positive chemical ionization mass spectra were generated at 90 eV by using ammonia as the reagent gas at a source pressure of 40 Pa. For this mode of operation, the source temperature was maintained at 90°C and the scanning rate was 0.7 s from 70 to 300 atom mass units.

Infrared spectra were determined in the gas phase with a Bruker model IFS85 Fourier transform infrared spectrometer coupled to a Carlo Erba model HRGC 5160 apparatus as described previously (18).

4-Hydroxydecanoic acid preparation. γ -Decalactone (5 g) was saponified in 100 ml of a KOH-methanol (15%, wt/vol) solution. After 2 h of refluxing, the solution was cooled with 100 ml of distilled water and washed twice with distilled pentane to remove the unsaponified phase. The organic phase was discarded, and the aqueous phase was concentrated under a vacuum by rotary evaporation to a final volume of 25 ml. The pH of this solution was adjusted to 8.0 with concentrated HCl. Then 5 g of CaCl_2 was added to the acidified solution, and the resulting precipitate was collected as described above. After the precipitate was redissolved in phosphate buffer, the solution was used for the toxicity experiments.

Data analysis. All data were obtained from triplicate assays. An analysis was performed by using SigmaPlot software (Jandel Scientific GmbH) for curve fitting, and QuattroPro software (Borland International, Inc.) and SAS software (28) were used for a statistical analysis of the data.

RESULTS

Growth and production of γ -decalactone. Figure 1 shows the viabilities of *Sporidiobolus salmonicolor* and *Sporidiobolus ruinenii* at various times in cultures producing γ -decalactone and the levels of lactone attained. Production of γ -decalactone began immediately after addition of the substrate (5% at 24 h), and the maximum values were reached after 130 h of growth for *Sporidiobolus salmonicolor* and after 216 h of growth for *Sporidiobolus ruinenii*. For both organisms, the maximum concentration of γ -decalactone achieved was the same. In contrast, during production of γ -decalactone the number of viable cells decreased markedly after 96 h in the *Sporidiobolus salmonicolor* culture, and the medium was sterile after 130 h of culture. Surprisingly, *Sporidiobolus ruinenii* did not seem to be affected by the decanolide, despite similar levels of production. Evidently the two organisms have different sensitivities to γ -decalactone. Acidification of the cultures to pH 2.0 prior to extraction increased the γ -decalactone concentration 2.5-fold in the *Sporidiobolus ruinenii* culture but made no difference in the yield in the *Sporidiobolus salmonicolor* culture.

The amounts of lactone in the different fractions of cultures

FIG. 1. Time course of cell viability ($\square$) and lactone recovery at pH 6 ($\circ$) and pH 2 ($\bullet$) during growth of cultures of *Sporidiobolus salmonicolor* (A) and *Sporidiobolus ruinenii* (B) in fermentors. Methyl ricinoleate (41 g/liter) was added at the time indicated by the arrow. The conditions used are described in the text.

are shown in Table 1. The distribution of lactone among the three fractions of a culture of *Sporidiobolus salmonicolor* revealed that a major amount (68%) of the decanolide was present in the oil fraction (i.e., methyl ricinoleate). Acidification of each phase before extraction of the lactone resulted in only slight increases in the amounts, and the distribution was the same at pH 2.0 as it was at pH 6.0. In contrast, the lactone content of a culture of *Sporidiobolus ruinenii* showed that the amounts of the decanolide in the oil and aqueous fractions were very similar at a neutral pH, but acidification prior to extraction resulted in 4.5-fold more lactone in the aqueous phase than in the oil phase (84% of the lactone was in aqueous fraction). This change in the distribution profile in the *Sporidiobolus ruinenii* culture was due to the presence in the aqueous

TABLE 1. Percentages of volume and amounts of lactone recovered before acidification (pH 6) and after acidification (pH 2) in the different fractions (yeast, oil, and aqueous fractions) obtained from 9-day-old cultures of *Sporidiobolus salmonicolor* and *Sporidiobolus ruinenii*

Fraction	Vol % ^a	<i>Sporidiobolus salmonicolor</i>		<i>Sporidiobolus ruinenii</i>	
		Lactone content (mg) at:		Lactone content (mg) at:	
		pH 6	pH 2	pH 6	pH 2
Aqueous	94.1	156 $\pm$ 18 ^b	200 $\pm$ 20	240 $\pm$ 13	1,066 $\pm$ 8
Oil	3.5	343 $\pm$ 49	400 $\pm$ 55	250 $\pm$ 2	212 $\pm$ 1.9
Yeast	2.4	5 $\pm$ 1.7	9 $\pm$ 1.9	31 $\pm$ 3	30 $\pm$ 3
Aqueous + CaCl_2 treatment					0

^a The total volume was 1 liter.

^b The values are the means $\pm$ standard deviations for three replicates.

FIG. 2. (A) Total ion current chromatogram of an ether extract obtained from the calcium salt recovered as described in Materials and Methods. Peak 1', γ -decalactone; peak 2', 4-hydroxydecanoic acid methyl ester; peak 3', heptanoic acid methyl ester; peak 4', 2-oxo-octanoic acid methyl ester; peak 5', 2-hydroxyoctanoic acid methyl ester; peak 6', 4-oxodecanoic acid methyl ester; peak 7', 6-hydroxydodecanoic acid methyl ester; peak 8', 6-hydroxydodecanoic acid methyl ester; peak 9', 8-hydroxytetradecanoic acid methyl ester; peak 10', ricinoleic acid methyl ester. (B) Mass spectrum of the compound identified as 4-hydroxydecanoic acid methyl ester. (C) Infrared spectrum of the compound identified as the 4-hydroxydecanoic acid methyl ester by gas chromatography-mass spectrometry analysis.

fraction at a neutral pH of a form of γ -decalactone which is not extracted by organic solvents. This compound might be the corresponding acid (4-hydroxydecanoic acid), but we cannot be sure. However, when calcium chloride was added to an aqueous fraction of a *Sporidiobolus ruinenii* culture and the resulting precipitate was removed, no lactone was recovered at pH 2 (Table 1). This shows that the precursor was precipitated as the calcium salt and indicates that a free carboxylic function is present on the molecule. To confirm this, a gas chromatography-mass spectrometry analysis was performed with the precipitate recovered and methylated as described in Materials and Methods. On the resulting total ion current chromatogram two relevant peaks were characterized (Fig. 2A). The first peak (peak 1') was identified as γ -decalactone (with the characteristic ion at m/z 85 attributed to the lactone ring). The second peak (peak 2') gave a mass spectrum (Fig. 2B) similar to the mass spectrum described by Umano et al. for 4-hydroxyoctanoic acid methyl ester (33), but it had no clear molecular ion. The peak with the highest mass found, at m/z 153, was interpreted as a result of water and methoxy group loss from the molecular ion. The molecular weight (202) was confirmed by performing a chemical ionization analysis with ammonia as the reagent gas. The presence of the major fragment ion at m/z 85 in the electron impact mass spectrum, a feature also present in the spectrum of 4-hydroxyoctanoic acid methyl ester (33), could be attributed to the cyclization of the hydroxyacid into a lactone ring during the analysis in the source of the mass spectrometer. This phenomenon was also observed in the chemical ionization experiment, in which the presence of an ion at m/z 188 could be explained by a pseudomolecular ion at $(M + 18)^+ = (M + NH_4)^+$ of γ -decalactone. The identity of 4-hydroxydecanoic acid was confirmed by a gas chromatography-Fourier transform infrared analysis (Fig. 2C). The gas phase infrared spectrum of the methyl ester exhibited characteristic features of an ester group (1,759 and 1,169 cm^{-1} for carbonyl and C-O-C absorption, respectively) and a hydroxy group (3,653 cm^{-1} for OH stretching absorption). The medium absorption band at 1,811 cm^{-1} was attributed to the carbonyl absorption of a γ -lactone ring, a common feature of all 4-hydroxy acid esters (20), resulting from cyclization in the heated measuring cell.

In Fig. 2A, other compounds that could have resulted from ricinoleic acid methyl ester (peak 10') catabolism were tentatively identified. These compounds are heptanoic acid methyl ester (peak 3'), 2-hydroxyoctanoic acid methyl ester (peak 5'), 6-hydroxydodecanoic acid methyl ester (peak 7'), 6-hydroxydodecanoic acid methyl ester (peak 8'), and 8-hydroxytetradecanoic acid methyl ester (peak 9'). Release of intermediates into the culture medium from yeast cells was described first by Okui et al. (25) in 1963 and more recently by Gatfield et al. for *Candida lipolytica* (12) and by Spinnler et al. for *Sporidiobolus ruinenii* (30). Moreover, 2-oxo-octanoic acid methyl ester (peak 4') and 4-oxo-decanoate acid methyl ester (peak 6') were also identified and could have come from catabolism of the intermediates. Attempts at definitive identification of these compounds are now in progress.

Toxicity of γ -decalactone and 4-hydroxydecanoic acid. As shown in Fig. 1, maintaining cell viability appears to be related not only to the level of production but also to the structure of

the molecule produced. To investigate the structure, the growth of the two species in the presence of γ -decalactone and 4-hydroxydecanoic acid was monitored. Figures 3A and A' show the effects of different γ -decalactone concentrations on the growth of *Sporidiobolus salmonicorum* and *Sporidiobolus ruinenii*. An analysis of variance (Student's Newman-Keuls test [28]) was used to test the significance of maximum specific growth rate (Table 2) differences when product concentrations were changed. The presence of lactone during batch growth of *Sporidiobolus* spp. resulted in an increase in the lag phase and significant inhibition of the maximum specific growth rate for both species. Moreover, no cell multiplication was observed at lactone concentrations of more than 100 mg/liter for *Sporidiobolus salmonicorum* and at lactone concentrations of more than 300 mg/liter for *Sporidiobolus ruinenii*, which seemed to be more tolerant to the lactone. This suggested that the MIC for *Sporidiobolus salmonicorum* was between 100 and 200 mg/liter and that the MIC for *Sporidiobolus ruinenii* was between 200 and 300 mg/liter. These values are lower than the levels of

FIG. 3. Growth kinetics of *Sporidiobolus salmonicorum* (A, B, and C) and *Sporidiobolus ruinenii* (A', B', and C') cultivated with different concentrations of γ -decalactone (A and A') and 4-hydroxydecanoic acid (B and B'). (C and C') 4-Hydroxydecanoic acid contents of cultures of *Sporidiobolus salmonicorum* (C) and *Sporidiobolus ruinenii* (C') grown in the presence of different concentrations of this acid. Symbols: $\bullet$, 0 mg/liter; $\square$, 100 mg/liter; $\blacksquare$, 200 mg/liter; $\circ$, 300 mg/liter; $\triangle$, 500 mg/liter. O.D. 620 nm, optical density at 620 nm.

TABLE 2. Effects of γ -decalactone and 4-hydroxydecanoic acid on maximum specific growth rates^a

Chemical	Concn (mg/liter)	Maximum specific growth rate (h ⁻¹) of:	
		<i>Sporidiobolus salmonicolor</i>	<i>Sporidiobolus ruinenii</i>
γ -Decalactone	0	0.27 $\pm$ 0.05 ^b	0.29 $\pm$ 0.04
	100	0.16 $\pm$ 0.01	0.18 $\pm$ 0.01
	200	0	0.17 $\pm$ 0.02
	300	0	0
	500	0	0
4-Hydroxydecanoic acid	0	0.26 $\pm$ 0.07	0.29 $\pm$ 0.03
	100	0.22 $\pm$ 0.01	0.27 $\pm$ 0.04
	200	0.21 $\pm$ 0.01	0.29 $\pm$ 0.07
	300	0.18 $\pm$ 0.03	0.26 $\pm$ 0.03
	500	0.13 $\pm$ 0.01	0.26 $\pm$ 0.03

^a See Fig. 3.^b The values are the means $\pm$ standard deviations for three replicates.

production shown in Fig. 1 but could be explained by trapping in the oil fraction (i.e., ricinoleic acid methyl ester phase) and consequent apparent protection of the yeast cells from inhibitory effects (Table 1).

Sporidiobolus salmonicolor (Fig. 3B) was able to grow in the presence of all of the 4-hydroxydecanoic acid concentrations used. However, the growth curves revealed an increase in the lag phase and a significant effect on the maximum specific growth rate, which was reduced to 0.13 h⁻¹ at a concentration of 500 mg/liter (Table 2). For *Sporidiobolus ruinenii* (Fig. 3B'), the results were quite different. The growth of this species was not affected by the presence of the acid over the range of concentrations tested. Measurements of the concentrations of 4-hydroxydecanoic acid during the growth of *Sporidiobolus salmonicolor* and *Sporidiobolus ruinenii* showed that the acid was totally catabolized by *Sporidiobolus ruinenii* (Fig. 3C') at all of the concentrations used. Surprisingly, the concentration of the acid did not change during growth of *Sporidiobolus salmonicolor* (Fig. 3C). A similar difference was observed for the two organisms in experiments performed with γ -decalactone (data not shown).

DISCUSSION

The main differences in ricinoleic acid metabolism between the two *Sporidiobolus* species are related to the production of 4-hydroxydecanoic acid. *Sporidiobolus salmonicolor* produces only the lactone form (i.e., γ -decalactone), while *Sporidiobolus ruinenii* produces a mixture of the lactone form and the free acid (i.e., 4-hydroxydecanoic acid). The physiological reasons which explain the differences between the two microorganisms are not clear. The differences could be due to a decrease in the pH of the broth, as observed elsewhere for *Tyromyces sambucus* (14). However, as the pH of each fermentation was controlled, the differences in our findings cannot be explained by acidification of the culture medium by *Sporidiobolus salmonicolor*.

The presence of the acid in the case of *Sporidiobolus ruinenii* might be due to the presence of a thioesterase that hydrolyzes the acyl coenzyme A esters after they are released from the β -oxidation system and the consequent accumulation of the different intermediates observed in the culture medium (Fig. 2A). However, the extent to which lactonization of the 4-hydroxydecanoic acid occurs enzymatically without acid catalysis is not clear (15). Additional in vitro investigations of the ox-

dation system of *Sporidiobolus* spp. will be required to clarify this question.

An alternative explanation of the results could be that γ -decalactone arises directly from the coenzyme A ester of 4-hydroxydecanoic acid. If this were the case, the presence of 4-hydroxydecanoic acid in *Sporidiobolus ruinenii* cultures would result from a selective ability of this yeast to open the lactone ring. 4-Hydroxydecanoic acid is water soluble and less toxic than lactone and could represent the product of the first step in lactone catabolism. A similar situation seems to have arisen in the work of Nago et al. (23), who studied the degradative pathway for massoialactone. The enzyme involved in ring opening was not characterized by these authors, but it may be a lipase since lipases have been used by other authors to cleave some lactones (5, 34). In 1994, Hadrich-Meyer and Berger (14) also described the localization of lipolytic and esterolytic activities in a γ -lactone-producing microorganism. An investigation of the lipase or esterase activities in *Sporidiobolus ruinenii* cultures is in progress.

The toxicity experiment (Fig. 3) showed that 4-hydroxydecanoate is less toxic for the microorganisms studied than γ -decalactone is. The toxicities of lactones were also described by Cutler et al. in 1986 (8) and by Hanssen and Urbasch in 1990 (16), who used 6-pentyl- α -pyrone. This lactone could inhibit the growth of bacteria (*Escherichia coli* and *Bacillus subtilis*) and some fungi. *Sclerotinia sclerotium*, *Fusarium oxysporum*, and *Trichothecium roseum* were inhibited by concentrations (0.1 to 0.7 mM) similar to the concentrations used in the present study (1.1 to 1.7 mM). Moreover, in 1993, Nago et al. (22) studied the ability of some microorganisms obtained from soil to grow on 2-deceno- δ -lactone. These authors showed that at a lactone concentration of 100 mg/liter the growth of bacteria was totally inhibited. They also isolated eight filamentous fungi which were able to grow on media containing 300 and 400 mg of lactone per liter. Four of these strains were able to produce lactone in biosynthetic pathways without additional fats or oil. This is not the case with *Sporidiobolus ruinenii*, which needs ricinoleic acid to produce γ -decalactone.

We have no data on the toxicities of some short-chain hydroxy fatty acids for yeasts. There are some previously published data for short-chain fatty acids (24, 35) which suggest that the presence of a hydroxyl group greatly decreases toxicity. We should note that the results obtained with *Sporidiobolus salmonicolor* did not necessarily indicate that there was a decrease in the maximum specific growth rate as a result of a high 4-hydroxydecanoate concentration. Because of the instability of 4-hydroxydecanoate at pH 6, some of this compound would have cyclized spontaneously with the formation of γ -decalactone. As we know, this compound is very inhibitory for *Sporidiobolus salmonicolor*. Another consideration is the inability of *Sporidiobolus salmonicolor* to catabolize 4-hydroxydecanoic acid even when the organism is in active growth.

Our findings are important because they suggest that providing a way of opening the lactone ring during the production of γ -decalactone could result in a way to overcome the toxicity of this compound for yeast cells. This could also prevent any further metabolism of the compound. Such a development could lead to production of much higher concentrations of product than the concentrations obtained previously and to a much more efficient industrial process for γ -decalactone production.

ACKNOWLEDGMENTS

We thank SBI, Boulogne, France, and Conseil Régional de Bourgogne, Dijon, France, for financial support of part of this work.

We thank C. Giniès and E. Sémon for measuring mass and Fourier transform infrared spectra. We especially thank Mark Dickinson, University of Hull, for corrections and advice.

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