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Sophorose lipid production from lipidic precursors: predictive evaluation of industrial substrates

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SUMMARY

Sophorose lipids stand out as biosurfactants with a wide potential for industrial application and which can be produced in good yield from glucose and a lipidic cosubstrate. *Candida bombicola* CBS 6009 (ATCC 22214) was used in the present study. The influence of the lipidic cosubstrate on various aspects of production performance of these glycolipids (final concentration, yield) and on product composition (in particular, the structure of the hydroxy fatty acid moieties) was assessed. An efficient fed batch technology was used. Lipidic cosubstrates which included *n*-alkanes, fatty acid methyl or ethyl esters, and vegetable and animal oils, markedly influenced product composition. In terms of production performance, the best substrates were oils or esters rich in C18:0 and C18:1 fatty acids. Optimal overall performance was obtained with esters (340 g L^{-1} sophorose lipids with rapeseed esters). Conclusions drawn from the results allow predictive evaluation of lipidic industrial substrates.

INTRODUCTION

Biosurfactants are a group of biomolecules that presently attracts attention. Among them, sophorose lipids produced by the yeast *Candida bombicola* stand out as a subject of particular interest. Sophorose lipids produced in fermentation broths are mixtures of compounds with related chemical structures. These compounds, which have been extensively studied [2,5,8,24], are presented in Fig. 1. They consist of a series of sophorose (2-*O*-β-D-glucopyranosyl-β-D-glucopyranose) derivatives, each linked to a hydroxy fatty acid. The sophorose derivatives identified, termed here structural classes, differ in that sophorose is lactonized (mainly in the 4″ position) or not, and acetylated or not in the 6′ and 6″ positions [2]. Ten structural classes have been identified [5]. Individual homologous compounds within each class differ from each other in their hydroxy fatty acid moiety, which varies in length (ranging mostly from C16 to C20), in the number of unsaturations (between 0 and 2) and in the position of the hydroxy group ($\omega-1$) or ω.

Biosurfactants have applications in agriculture, cosmetics, and the petroleum industry [7,9,15,17,26]. For sophorose lipids, specific applications mainly depend on the appropriate conjunction of several physical properties of the sophorose lipid mixture. As pointed out by Inoue et al. [13], these properties, such as solubilities in organic solvents or in water, or surface active properties, are related to the abundance of the different structural classes in the mixtures. Thus, some applications have been claimed specifically either for lactonic forms, which are, for example, bacteriostatic, [16] or for acidic ones which can act as moisturizing agents [1,20]. Some applications are more closely linked to the fatty acid moieties, e.g. in the cosmetic field [10]. Consequently, a large survey of all the potential applications requires detailed determination of individual sophorose lipids in the mixtures produced and makes it highly desirable to be able to control the composition of the fermentation products.

An important fact is that the possibility to produce sophorose lipids in very large amounts has been demonstrated [6]. High production performance is obtained when a fed batch technology involving two carbon sources, glucose and a lipidic substrate, is used. Some studies have also shown the existence of a relationship between the nature of the lipidic carbon source and the overall composition of the hydroxy fatty acid moiety of sophorose lipids produced [14,18,19,22].

For these reasons, establishing a relationship between the composition and the production performance of sophorose lipids on the one hand and the nature of the industrial lipidic cosubstrate used on the other, constitutes an important goal. In the present work, various criteria of sophorose lipid production performance (final concentration, yield) as well as product composition, were assessed under controlled fermentation conditions. Lipidic cosubstrates tested included individual compounds and mixtures: alkanes, fatty acid esters, vegetable and animal oils. Characterization of the fermentation products was performed with analytical tools previously developed [5], in particular by gradient HPLC, which allows for separation of the individual sophorose lipids.

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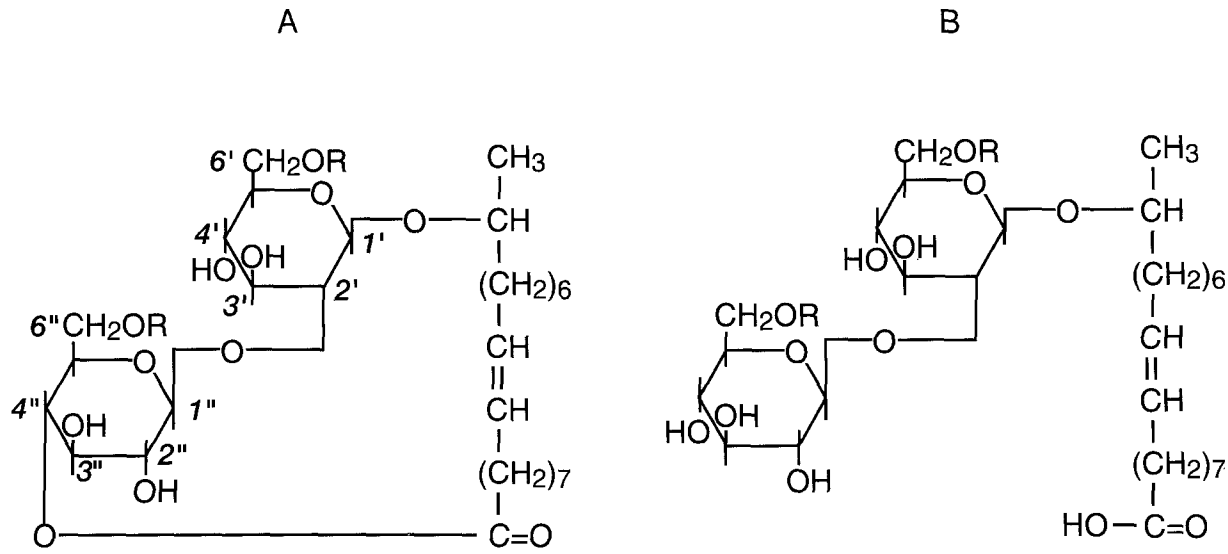

Fig. 1. Structural classes of sophorose lipids. Main classes are represented for the case of the usually most abundant individual compound where the constitutive hydroxy fatty acid is 17-hydroxy octadecenoic acid (17-hydroxy oleic). R=H or CH_3CO. (A) 1′,4″-lactone classes; (B) acid classes. In addition to the 1′,4″-lactone classes represented, 1′, 6′- and 1′, 6″-lactone classes have also been detected [2].

MATERIALS AND METHODS

Culture conditions

Candida bombicola CBS 6009 (ATCC 22214) was cultivated as previously described [6]. Briefly stated, cultures were carried out aerobically on a mineral medium supplemented with 5 g L^{-1} of dried cornsteep liquor at 25 °C in a 4-L fermentor. One of the two carbon sources was glucose which was supplied in excess from the beginning to the end of the culture by daily additions of 50 g L^{-1}. The second carbon source or cosubstrate (*n*-alkane or oil or fatty acid esters) was fed continuously. The feeding rate was chosen as non-limiting but so as to avoid large cosubstrate accumulation (the concentration in the fermentor was always below 15 g L^{-1}). During the fermentation, the pH was maintained at 3.5 by automatic addition of NaOH. The aeration rate (0.5 v.v.m.), the dissolved O_2 concentration in the culture medium and the CO_2 in the output gas were monitored.

The cosubstrates used were from commercial sources. Vegetable oils were obtained from Robbe (Compiègne, France). Corresponding methyl and ethyl esters were obtained from these oils by transesterification in alkaline conditions. Methyl esters were used in the case of sunflower, palm and linseed oils. Ethyl esters were used in the case of rapeseed oil. Alkanes were from Sigma (St Louis, MO, USA).

Analytical procedures

Cell biomass was estimated by dry weight, residual glucose by enzymatic analysis and residual lipidic cosubstrate by gas chromatography (GC) after toluene extraction and conversion into esters [6]. Alkanes were measured directly by GC after toluene extraction. Sophorose lipids were quantified after decanting the sophorose lipid layer from the yeast-containing aqueous phase of the broth and after washing with water. The water content of the sophorose lipid preparation was determined by loss of weight after lyophilisation [5].

TABLE 1

Performances of sophorose lipid production in cultures with *n*-alkane cosubstrates

n-alkane feedstock	Performances			
	Production[a] (g L^{-1})	Yield[a] (g g^{-1})	E_G[a] (mol mol^{-1})	E_L[a] (mol mol^{-1})
None[b]	20	0.06	0.06	
C12	17	0.07	0.09	0.06
C14	20	0.08	0.12	0.06
C16	95	0.32	0.32	0.50
C18	175	0.33	0.36	0.46

[a] See definition in Materials and Methods.
[b] Sophorose lipid production on glucose only.

The glucose moiety of sophorose lipids was quantified by the anthrone method [11]. The other methods mentioned below, used for sophorose lipid analysis, were described by Davila et al. [5].

The lipidic moiety (hydroxy fatty acids) was released by transesterification of sophorose lipids by H_2SO_4-methanol for 1 h at 100 °C. A good reproducibility in GC analysis was achieved by silylation of the hydroxy group with Sylon TP (Supelco, Bellefonte, PA, USA). The silylated hydroxy fatty acid methyl esters were analysed by GC using a 0.32 mm × 30 m DB-5 column (J & W Scientifics, Folsom, CA, USA) with programmed temperature (from 150 to 290 °C at a rate of 4 °C min^{-1}). Identification of hydroxy

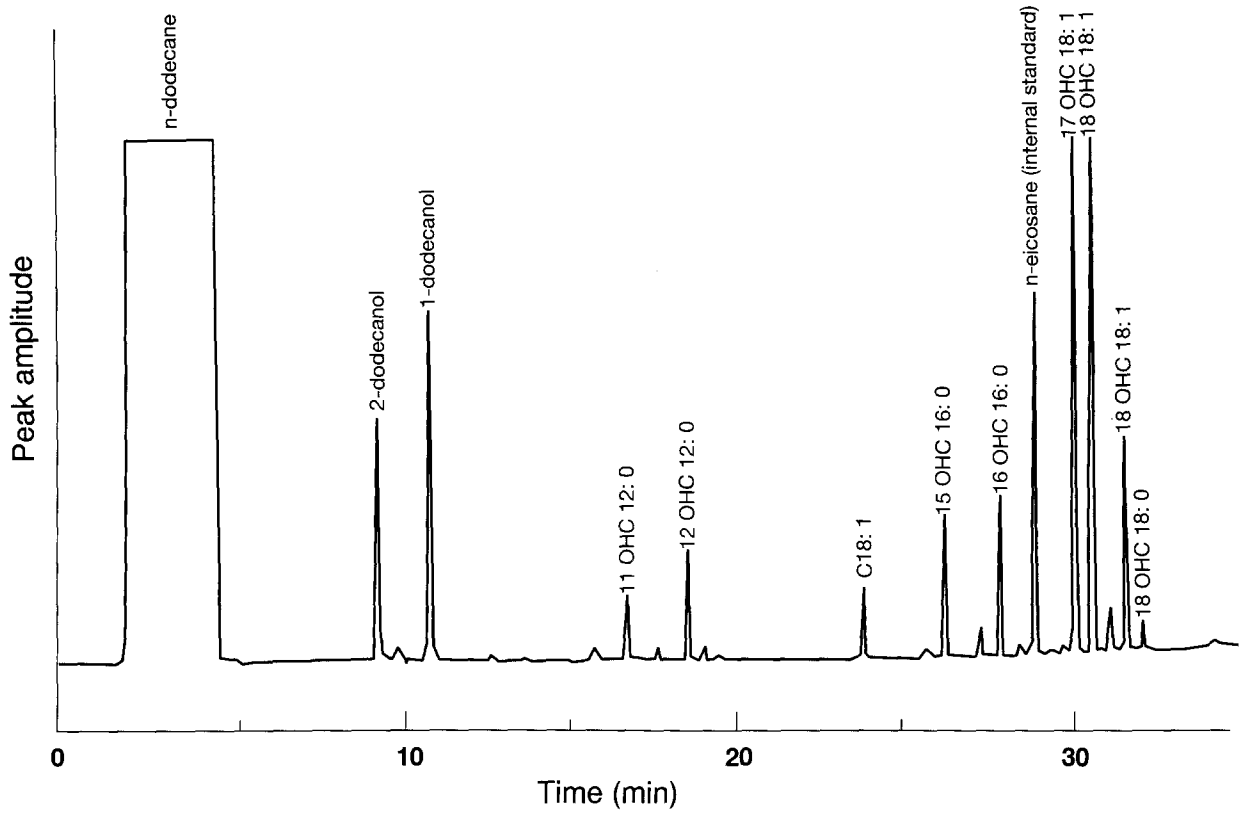

Fig. 2. Lipidic composition of sophorose lipids: gas chromatogram of the lipidic moieties resulting from methanolysis of sophorose lipids produced from *n*-dodecane and glucose. The conditions were as indicated in Materials and Methods. Remaining *n*-dodecane was eluted within the solvent front.

TABLE 2

Hydroxy acid composition of sophorose lipids produced from *n*-alkane cosubstrates (%)

Hydroxy acid moieties	*n*-alkane feedstock				
	None	C12	C14	C16	C18
11-OH C12:0		1.9			
12-OH C12:0		2.1			
13-OH C14:0			7.8		
14-OH C14:0			<0.1		
15-OH C16:0	13.1	4.2	10.0	33.2	2.4
16-OH C16:0	14.5	4.6	10.8	44.3	2.1
15-OH C16:1		<0.1	<0.1		
16-OH C16:1		1.4	6.3		
17-OH C18:0	38.4	16.3	8.8	4.5	74.7
18-OH C18:0	<0.1	0.9	<0.1	<0.1	3.7
17-OH C18:1	29.0	29.2	32.8	5.4	10.2
18-OH C18:1	5.0	6.1	5.7	<0.1	1.6
Others		3.8	3.7	10.4	2.3

TABLE 3

Performances of sophorose lipid production in cultures with oil or ester cosubstrates

Oil or ester feedstock	Performances			
	Production ($g\ L^{-1}$)	Yield ($g\ g^{-1}$)	E_G ($mol\ mol^{-1}$)	E_L ($mol\ mol^{-1}$)
Rapeseed esters	340	0.65	0.70	0.79
Rapeseed oil	255	0.53	0.58	0.70
Sunflower esters	235	0.52	0.51	0.70
Sunflower oil	172	0.43	0.44	0.58
Palm esters	240	0.67	0.67	0.92
Palm oil	82	0.39	0.35	0.62
Linseed esters	122	0.25	0.20	0.37
Fish oil	51	0.21	0.15	0.39

fatty acid methyl esters was performed by coupling GC to mass spectrometry.

Separation of the individual sophorose lipids from fermentation product was achieved by gradient elution HPLC using a Hypersil C18, 5-μm (4.6 mm × 150 mm) column from Interchim (Montluçon, France). The flow rate was 1 ml min^{-1}

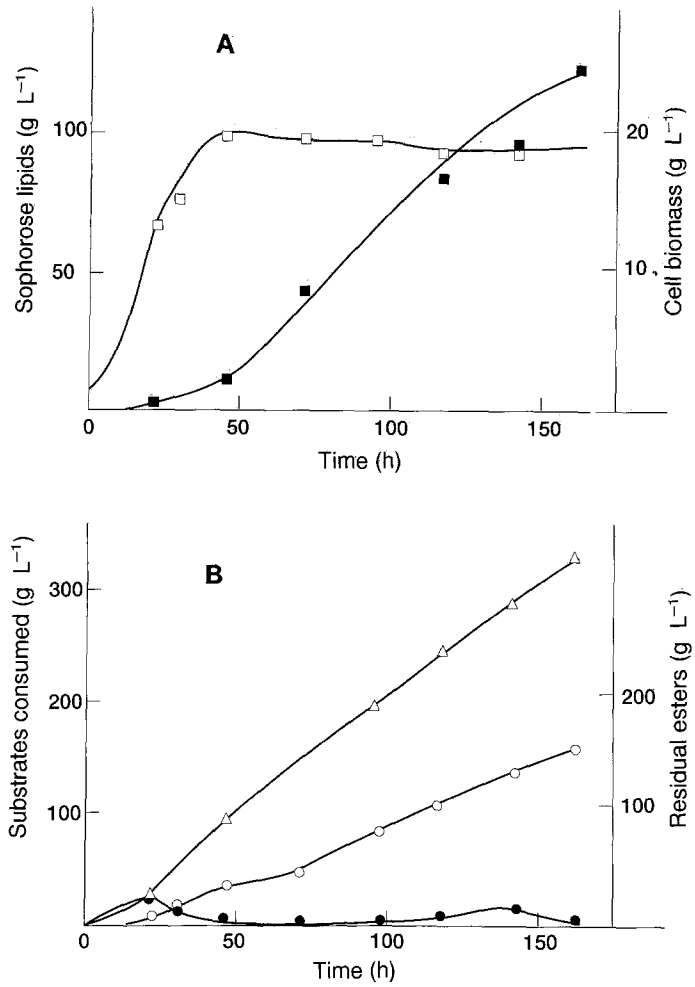

Fig. 3. Sophorose lipid production by *Candida bombicola* in fed-batch culture on linseed esters and glucose. (A) Time course of growth and sophorose lipid production. (B) Time course of substrate consumption. The conditions were as indicated in Materials and Methods. □, biomass produced; ■, sophorose lipids produced; △, glucose consumed; ○, linseed esters consumed; ●, remaining linseed esters.

and the composition of the acetonitrile–water eluent was programmed from 2% to 70% acetonitrile in 48 min. For detection, an evaporative light-scattering (ELS) detector (mass detector) DDL 21 from Cunow (Cergy Saint Christophe, France) was used.

Data acquisition in liquid and gas chromatography was done with HP 1000/A600 equipment (Hewlett-Packard, Sunnyvale, CA, USA).

Formulation of fermentation results

Final production of dry sophorose lipids (in g) was estimated after 165 h of incubation and expressed by litre of initial volume. Yield (Y) was calculated as the ratio of the mass of the sophorose lipids produced, Sl (in g), to the sum of the masses of glucose, Gc (in g), plus lipidic cosubstrate consumed, Lc (in g). Y = Sl(g)/[Gc(g) + Lc(g)].

Efficiency for glucose incorporation into sophorose lipids (E_G) was defined as the ratio of glucose incorporated into sophorose lipids, equal to 2 Sl (in mol), to glucose consumed, Gc (in mol). E_G = 2 Sl (mol)/Gc(mol). Efficiency for alkane or fatty acid incorporation, E_L, was the molar ratio of hydroxy fatty acid of sophorose lipids, Sl (in mol), to consumed lipidic cosubstrate, Lc (in mol). E_L = Sl (mol)/Lc(mol). The ω-hydroxylation ratio, Hω, was calculated for each hydroxy fatty acid as the ratio of the ω form (W) to the sum of ω form (W), plus $\omega-1$ form (W−1). Hω = W/[W + (W−1)].

RESULTS

Utilization of alkanes

The influence of the number of carbons of the long chain cosubstrate was determined. For this purpose sophorose lipids were produced from glucose and from a *n*-alkane as lipidic precursor. Alkanes tested had an even number of carbons between 12 and 18. The performances obtained for these fermentations are shown in Table 1. In each case, sophorose lipid production was determined at the end of fermentation. The results in Table 1 show that production increased with the number of carbons of the lipidic cosubstrate tested. Yields of sophorose lipids from both carbon sources were also calculated. The same values were obtained for fermentations on *n*-C12 and *n*-C14 alkanes. Performances from fermentations on *n*-C16 and *n*-C18 alkanes were several-fold higher than those from fermentations on *n*-C12 and *n*-C14 alkanes.

Lipidic compositions of sophorose lipids obtained from alkanes were determined qualitatively and quantitatively by gas chromatography after acid hydrolysis. As shown in Fig. 2, hydroxy fatty acids were discriminated according to their number of carbons, their degree of unsaturation and the location of their hydroxy group. Lipidic compositions are presented in Table 2. Lipidic moieties of sophorose lipids mainly consisted of hydroxy fatty acids although long chain alcohols were also detected as illustrated in Fig. 2. The existence of these long chain alcohols as lipidic moieties of sophorose lipids and not as free alcohols was indicated by the fact that they were not extracted by hot hexane from sophorose lipids. These compounds have already been detected by Tulloch and Spencer [23]. Free octadecenoic acid was also observed in alkane fermentation broths. Analysis of the products obtained (Table 2) shows that the nature of lipidic precursors largely influenced hydroxy fatty acid composition. For fermentations on *n*-C16 and *n*-C18 alkanes, the cosubstrates appeared to be directly incorporated in hydroxy acid moieties without any changes in the carbon chain. For shorter cosubstrates, some hydroxy acids were produced by direct conversion but a larger portion was formed after chain elongation by 2, 4 or 6 carbons. Desaturation also appeared to occur in these fermentations for C16 and C18 fatty acids, in agreement with the report of Brett et al. [3] on the presence of desaturases in *C. bombicola* extracts. The lower performances in the fermentations of *n*-C12 and *n*-C14 alkanes could probably be accounted for by the requirement for alteration of such

TABLE 4

Hydroxy fatty acid composition of sophorose lipids produced from oil or ester cosubstrates

Oil or ester feedstock	Hydroxy acids (%)						
	15-OH C16:0	16-OH C16:0	17-OH C18:0	17-OH C18:1	18-OH C18:1	17-OH C18:2	18-OH C18:2
None	13.1	14.5	38.4	29.0	5.0	<0.1	<0.1
Rapeseed esters	0.8	1.7	2.5	67.8	12.0	4.9	10.3
Rapeseed oil	1.3	1.8	2.0	69.1	11.9	5.3	8.6
Sunflower esters	2.2	2.6	7.5	19.9	4.0	19.9	43.9
Sunflower oil	2.1	2.3	8.7	33.4	4.5	16.5	32.5
Palm esters	17.2	20.7	7.2	38.1	6.6	3.4	6.8
Palm oil	15.8	16.0	12.9	41.9	5.9	2.8	4.7
Linseed esters	6.0	7.3	13.0	35.6	6.9	9.0	22.2
Fish oil	13.7	13.0	15.6	48.0	6.0	1.5	2.0

cosubstrates before their incorporation into the hydroxy acid moiety.

Location of the hydroxy group is another characteristic of fatty acids of sophorose lipids. The hydroxy group could be borne by the terminal (ω) or subterminal ($\omega-1$) carbon. The data in Table 2 allow one to calculate the ω-hydroxylation ratio, Hω, for each fatty acid chain encountered in alkane fermentations. The higher the number of carbons of the fatty acid chain, the more the ω-hydroxylation ratio decreased (Hω around 0.5 for C16:0 and 0.05 for C18:0 hydroxy fatty acids). For an identical number of carbons, the ω-hydroxylation ratio was higher for the unsaturated fatty acids than for their saturated homologs (Hω of 1.0 for C16:1 versus 0.5 for C16:0 hydroxy fatty acids). The site specificity of hydroxylation seems to be related to fatty acid chain length. These results are in agreement with those published by Tulloch et al. [21,25]. They illustrate the proposal of these authors that site specificity of hydroxylation is governed by fatty acid chain length, taking into account that the length of fatty acid molecules is decreased by the presence of double bonds.

TABLE 5

Fatty acid composition of oil or ester cosubstrates

Oil or ester feedstock	Fatty acids (%)				
	C16:0	C18:0	C18:1	C18:2	C18:3
Rapeseed esters	5.0	1.8	61.2	22.0	10.0
Rapeseed oil	4.9	1.5	60.1	23.1	10.4
Sunflower esters	6.5	4.7	17.1	71.7	<0.1
Sunflower oil	6.8	4.9	22.8	65.5	<0.1
Palm esters	44.2	7.3	38.2	10.3	<0.1
Palm oil	43.2	5.6	39.7	11.5	<0.1
Linseed esters	5.9	4.4	20.2	15.9	53.6
Fish oil[a]	20.5	3.1	17.4	<0.1	<0.1

[a] Other fatty acids present: C14:0, 8.5%; C16:1, 7.8%; C18:4, 4.2%; C20:1, 5.8%; C20:4, 15.7%; C22:1, 4.7%; C22:5, 2.2%; C22:6, 10.1%.

TABLE 6

Incorporation of fatty acids from oil or ester cosubstrates

Oil or ester feedstock	Efficiency for incorporation of fatty acids, E_L (mol mol^{-1})				
	C16:0	C18:0	C18:1	C18:2	C18:3
Rapeseed esters	0.44	1.21	1.14	0.60	<0.2
Rapeseed oil	0.44	0.98	0.96	0.48	<0.2
Sunflower esters	0.48	1.05	0.92	0.59	
Sunflower oil	0.37	1.01	0.95	0.43	
Palm esters	0.81	0.94	1.11	0.94	
Palm oil	0.51	1.10	0.84	0.45	
Linseed esters	1.14	1.50	1.07	0.99	<0.2
Fish oil	0.50	1.93	1.20	>1	

Composition of sophorose lipids with respect to their structural classes was determined by HPLC with isocratic elution. The most abundant structural classes detected were those usually described [2]. We noticed an exceptionally high content (up to 85% of diacetylated lactones in sophorose lipids produced on *n*-C16 and *n*-C18 alkanes. For fermentations on *n*-C12 and *n*-C14 alkanes, sophorose lipids produced were distributed into the different structural classes (data not shown).

Utilization of oils and esters

Results from fermentations on individual *n*-alkanes were used as a basis for the study of cosubstrate mixtures

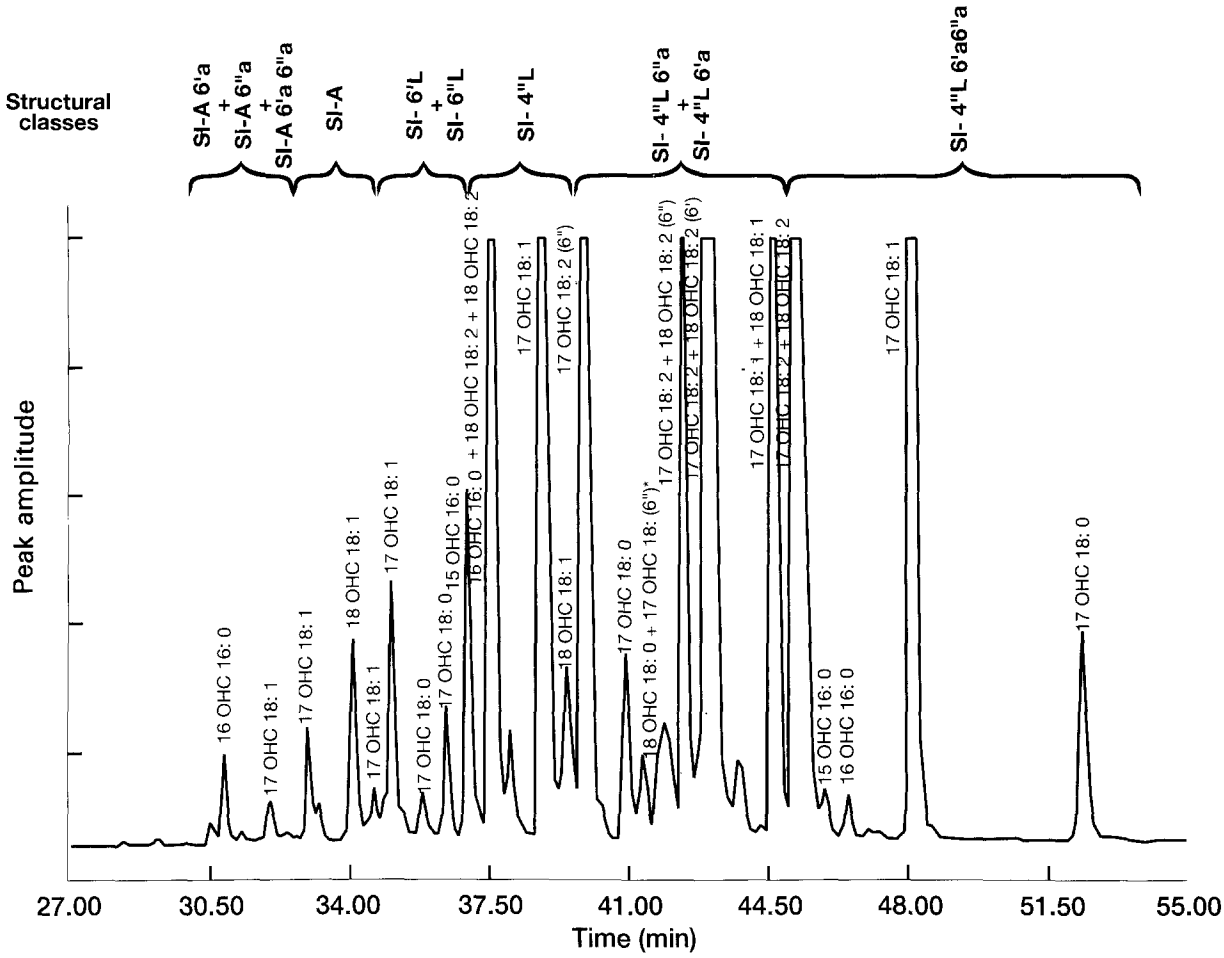

Fig. 4. Resolution into individual sophorose lipids of fermentation mixtures by gradient elution HPLC. Example of the sophorose lipids from sunflower esters and glucose. Notation according to Davila et al. [5]: Sl, sophorose lipids; L, lactone; A, acid; a, acetyl. Individual sophorolipids are designated by adjunction of the notation for the constitutive hydroxy fatty acid: Sl-(17-OH C18:1) 4"L6'a 6"a stands for (17-hydroxyoctadecenoic) 1',4"-lactone 6',6" diacetate sophorolipid.

TABLE 7

Localization of hydroxylation sites in sophorose lipids produced from oil or ester cosubstrates

Oil or ester feedstock	ω-hydroxylation ratio for hydroxyacids, Hω			
	C16:0	C18:0	C18:1	C18:2
Rapeseed esters	0.68	0	0.15	0.68
Rapeseed oil	0.58	0	0.14	0.62
Sunflower esters	0.54	0	0.17	0.69
Sunflower oil	0.52	0	0.12	0.66
Palm esters	0.54	0	0.15	0.66
Palm oil	0.50	0	0.19	0.62
Linseed esters	0.50	0	0.16	0.71
Fish oil	0.49	0	0.11	0.58

susceptible to be used as sophorose lipids precursors in industrial fermentations. These cosubstrates were fatty acid glycerides such as triglycerides of oils (rapeseed, sunflower, palm or linseed) and corresponding esters obtained by transesterification. Methyl or ethyl esters were found to be similar, in the case of rapeseed, with regard to fermentation kinetics of sophorose lipid production (data not shown). Sophorose lipids were also produced from fish oil.

During fermentation, the lipidic cosubstrate was fed at a suitable constant rate as indicated in Materials and Methods whereas the other carbon source (glucose) was supplied in excess by stepwise additions. Detailed kinetics of a fermentation carried out with glucose and linseed methyl esters are presented in Fig. 3(A) and (B). As shown in Fig. 3(A), growth started after a short lag phase and slowed down after about 30 h. At that time, sophorose lipid production began. Glycolipid production was dissociated from growth. The time course of substrate consumption is shown in Fig. 3(B). Glucose consumption began in the early phase of culture and largely supported growth. Uptake of linseed esters, which was low during this period, increased

TABLE 8

Distribution of sophorose lipids produced from oil or ester cosubstrates into structural classes

Oil or ester feedstock	Distribution in structural classes[a] (%)				
	Non-acetylated acids	Acetylated acids	Non-acetylated lactones	Mono-acetylated lactones	Diacetylated lactones
Rapeseed esters	9.4	18.6	17.7	3.2	51.1
Rapeseed oil	6.9	17.2	8.4	6.0	61.5
Sunflower esters	6.5	37.0	14.6	26.4	15.5
Sunflower oil	13.0	31.4	10.8	2.7	42.1
Palm esters	12.1	8.8	16.6	23.0	39.5
Palm oil	9.6	16.0	12.7	2.5	59.2
Linseed esters	21.5	17.0	8.4	20.9	32.1

[a] See Fig. 1.

during sophorose lipid production. Then, glucose and esters were consumed at a constant ratio. During the experiment, residual esters were partially hydrolysed into fatty acids which appeared to be inhibitory for sophorose lipid production (data not shown). As a consequence, the feeding rate of linseed esters was chosen as non-limiting while minimizing the concentration of residual cosubstrate in the medium. When fatty acid accumulation was observed during the production stage (at 140 h) ester feeding was interrupted.

Performances obtained from esters and oils are shown in Table 3. Sophorose lipid productions observed were rather high compared to previously published data [4,12,27], rapeseed ethyl esters yielding the best results. The relatively modest performances obtained from linseed esters and fish oil can be related to lower incorporation of constitutive fatty acids in sophorose lipids. In the case of rapeseed, sunflower and palm oils, enhanced productions were obtained from esters that are easily hydrolysed, thus providing an available lipidic precursor and energy source. In the case of oils, hydrolysis of the glycerides appears to be the limiting stage of sophorolipid production.

The hydroxy fatty acid composition of glycolipids was determined (Table 4) and compared to the fatty acid composition of oils and esters (Table 5). Table 4 shows that in the case of rapeseed, sunflower and palm oils, the hydroxy acid compositions of sophorose lipids obtained from esters and oils were similar. They appeared to be largely influenced by fatty acid composition of the cosubstrates. Fatty acids with 16 or 18 carbons were directly incorporated into the hydroxy acid fraction without any alteration of their lipidic chain. These results are in agreement with those obtained using *n*-alkanes. However linolenic acid (C18:3), which is the major constituent of linseed esters (over 50%) and is also present in rapeseed, did not follow this rule as corresponding hydroxy fatty acids were not detected. Hydroxy acid composition determined in sophorose lipids from fish oil confirmed that fatty acids shorter than palmitic acid were not incorporated in glycolipids. The same situation held for fatty acids longer than stearic acid. Again, fatty acids containing more than two double bonds were not incorporated whatever the length of the molecule. Fatty acids not directly incorporated into sophorose lipids were oxidized into CO_2 or could probably be used for sophorose lipids synthesis after suitable chain alterations.

The efficiencies for incorporation of fatty acids from lipidic cosubstrates into sophorose lipids are shown in Table 6 and provide a quantitative illustration of these observations. The highest rates of incorporation were observed for oleic and stearic acids, followed by palmitic and linoleic acids. This explains the high performance obtained with esters of rapeseed (rich in oleic acid) compared to those obtained with sunflower (rich in linoleic acid) and palm (rich in palmitic acid).

Table 7 shows the localization of the hydroxylation site of sophorose lipid hydroxy acids. Site specificity of hydroxylation appears to be associated with fatty acid chain length whatever the nature of the lipidic cosubstrate. These results are in agreement with those previously reported for *n*-alkanes.

Sophorose lipids from oil and esters cosubstrates were analysed for their distribution into structural classes. Gradient elution HPLC with evaporative light scattering detection was used [5]. The chromatographic profile obtained with sunflower ethyl esters as the lipidic cosubstrate is presented in Fig. 4. This profile gives the distribution into structural classes and into individual sophorose lipids. Results concerning structural classes are presented in Table 8. They provide evidence that the repartition of glycolipid structural classes is dependent on the nature of the lipidic cosubstrate used. Quite different compositions were obtained from oils and corresponding esters. Sophorose lipids produced from oils always exhibited a higher level of diacetylated lactones than sophorose lipids produced from esters. Sophorose lipid repartition into structural classes was also influenced by the fatty acid composition of the lipidic cosubstrate. Increased levels of acidic classes of sophorose lipids were obtained

from cosubstrates predominantly composed of polyunsaturated fatty acids (sunflower, linseed). In contrast, when lipidic cosubstrates were composed of stearic acid or oleic acid, lactonic classes were predominant and the structural class of diacetylated lactones was always the major component of the mixture of sophorose lipids. Thus, the distribution of structural classes of sophorose lipids appears to be dependent on the ability of the yeast to incorporate the fatty acids composing the lipidic cosubstrate.

DISCUSSION

The results presented above, obtained under standardized optimized fermentation conditions, allow comparative evaluation of the characteristics of sophorose lipid production in relation to the nature of the lipidic cosubstrate.

The best performance of sophorose lipid production in terms of yield and productivity was usually obtained with lipidic cosubstrates of appropriate chain length which were most efficiently incorporated in sophorose lipids as proposed by Tulloch et al. [21,25]. The case of polyunsaturated fatty acids, which were not incorporated, as shown in our study, even when their length would seem appropriate, constituted an exception, possibly because of their particular spatial configuration.

The portion of the lipidic cosubstrate, incorporated into sophorose lipids (E_L) amounted to 65% in the case of an excellent cosubstrate such as rapeseed esters [6]. The non-incorporated portion (35% in this favourable case) was mainly oxidized to CO_2. Fatty acids, which were less susceptible to direct incorporation into sophorose lipid hydroxyacids, could be modified. They were also more subject to oxidation to CO_2 which was, in some cases, quite extensive. This was also illustrated for example for linseed oil and fish oil. These cosubstrates contained polyunsaturated fatty acids which were degraded, bringing the portion of the cosubstrates oxidized into CO_2 up to about 70% as calculated from the fermentation balance.

The incorporation efficiencies, E_L, obtained for alkanes were comparable to those reported by Spencer et al. [19]. For esters, comparison of incorporation efficiencies with those of the literature was not possible since mixtures were used in our study, but the results are in agreement with the trends observed in the case of individual cosubstrates.

No information on the influence of the lipidic cosubstrate on the distribution of the sophorose lipid structural classes was previously available in the literature. The present study provides basic data concerning the identification of lipidic carbon sources suitable for the production of sophorose lipids with a defined composition.

The cosubstrates yielding the best production performances are oils or esters rich in C18:1 and C18:0 fatty acids which, unlike alkanes, do not require an initial step of hydroxylation. Terminal or subterminal hydroxylation of these fatty acids was found to be dependent on the length of the cosubstrate molecule as observed by Tulloch et al. [21,25]. Use of esters resulted in optimal overall performance and their use contributed to the very high production reported by Davila et al. [6]. The results presented allow for a good forecast of the production performance and of the product composition which can be expected from a given lipidic cosubstrate, thus providing very useful information for the selection of the most appropriate industrial raw materials for sophorose lipid production.

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