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SOPHOROSE LIPIDS PRODUCED FROM SUCROSE

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SUMMARY

Torulopsis bombicola produced sophorose lipids when growing on sucrose in similar yields as if growing on glucose. Besides yield the composition of sophorose lipids characterized by TLC/FID was greatly influenced by yeast extract concentration in the shake flasks in contrary to highly aerated fermenter.

INTRODUCTION

Torulopsis bombicola produces a mixture of sophorose lipids during growth on glucose and/or oil substrates. Recently, Asmer et al. (1988) characterized the sophorose lipids produced on a mixture of glucose and oleic acid and on oleic acid alone. When glucose was a part of the medium, 50 - 60 % of 17-L-([2′-O-β-D-glucopyranosyl-β-D-glucopyranosyl]-oxy) -octadecanoic acid 1,4′-lactone 6′,6′′ diacetate was produced. Other lipidic derivatives of sophorose (SLs) that differ in the acetyl content, in the lactone function, and in fatty acid structure, have been found in isolates from different cultivations (Asmer et al., 1988; Inoue, 1988; Cooper and Paddock, 1984; Gorin et al., 1961; Tulloch et al., 1962).

Considering an application of SLs a major difference in properties of particular SL is connected to the presence of 6′,6′′ acetyl groups in a molecule. SL-1 is poorly soluble in water but more soluble non-acetylated SLs are to be preferred for some applications, especially in enhanced oil recovery. Asmer et al. (1988) reported that a ratio of glucose to the second carbon source (oil, fatty acids) of about 3/1 favoured the overproduction of SL-1. Lower ratio led to more hydrophilic sophorose lipids.

As glucose is rather rarely found in larger quantities in wastes we investigated the substitution of glucose by disaccharides, especially sucrose. Preliminary investigation of the influence of cultivation conditions on the composition of the SL mixture was also the aim of this study.

MATERIALS AND METHODS

The yeast *Torulopsis bombicola* ATCC 22214 was maintained on YM agar slants and cultivated on medium containing 0.1 % KH_2PO_4, 0.5 % $MgSO_4.7H_2O$, 0.01 % $FeCl_3$, 0.01 % NaCl was supplemented by yeast extract (YE, Difco), sugar (Sigma) and oil substrate in the concentrations described later. Sunflower oil used was of food grade without additives. Initial pH of media was 4.5±0.1. Flask experiments were carried out with 100 ml of media in 500 ml Erlenmayer flasks for 6 days (rotary shaker New Brunswick G-25R, 250 rpm, 30 °C). Inoculation was made by a platinum loop from agar slants. In case of cultivation in 1 l Bellco jar fermenter (700 ml of a medium, 350 rpm, 2 vvm of air), a whole agar slant rinsed with water was used for inoculation. Temperature and initial pH was the same as in case of flask experiments. There was no adjustment of pH during cultivation and pH decreased towards the end of cultivation to between 3.2 and 3.4. Medium was always sterilized with all components together at 121 °C for 20 min.

For estimation of biomass 5 ml of cultivation medium was diluted with 10 ml of water and centrifuged (2000 g). Pellets were resuspended in water and dried at 105 °C for 24 hours.

Sophorose lipids were extracted from 5 ml of cultivation broth by addition of 10 ml of ethyl acetate, thoroughly shaking and centrifuging (2000 g). TLC (10 ml of extract) was done on Bakerflex sheets Silica gel IB2-F (J.T. Baker) in the developing system $CHCl_3/CH_3OH/H_2O$ = 65/15/2 (v/v/v) and SLs were detected by α-naphthol. Two methods were used for quantitative estimation, TLC/FID using Chromatorods II that were developed in $CHCl_3/CH_3OH$ = 90/4 (v/v) and scanned by Iatroscan TH 10 (Iatron Laboratories, Japan), and dry weight of SLs according to Cooper and Paddock (1984). The latter method was used at the end of cultivation because it demands larger sample volume (50 ml). Iatroscan was employed for the estimation of SLs offering an advantage of very small amounts of samples needed for TLC/FID and complete separation of SLs from oil. Partial separation of SLs enabled limited characterization of produced SLs. Peaks of Sls on TLC/FID were identified by comparison of TLC and TLC/FID of different fractions after partial separation on a SIP-PAK Silica cartridge (Waters). 0.5 ml of sample of isolated SLs in ethyl acetate was applied on SIP-PAK cartridge, washed with hexane and eluted by TLC solvent mixture. The calibration of FID detector was done by measuring a set of different SLs isolates, correcting for fat content and calculating an average. TLC/FID allows to distinguish between highly hydrophobic diacetyl-SLs (SL-1) and more hydrophillic groups of monoacetyl-SL and/or SL without acetyl, on which basis the two SL groups were quantified.

Glucose was estimated in the water phase after extraction of SLs by the DNS method.

RESULTS AND DISCUSSION

To verify the possibility of substituting glucose by sucrose, the cultivations in shake flasks were run for 6 days. Table I shows that the results of cultivations on both substrates were similar. In general, YE concentration above 0.25 % reduced SLs yields especially on the sucrose media.

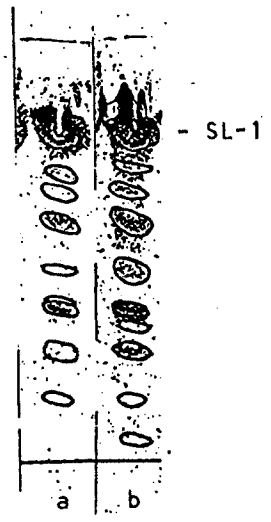

FIGURE 1 TLC of sophorose lipids isolated from the culture of *Torulopsis bombicola* growing on 10 % of sunflower oil, 1 % of YE and a) 10 % of glucose (300 μg of sophorose lipids in 10 μl); b) 10 % sucrose (500 μg of sophorose lipids in 10 μl).

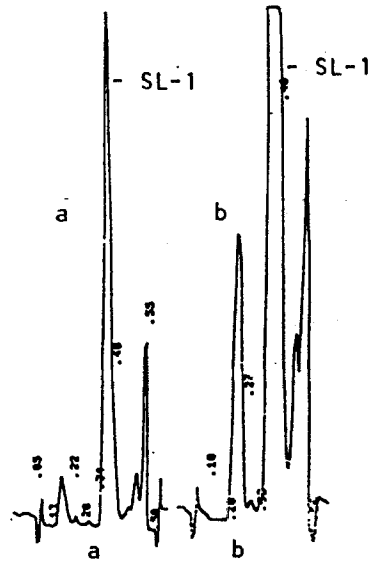

FIGURE 2 TLC/FID records of sophorose lipids isolated from the culture of *Torulopsis bombicola* growing on 10 % of sunflower oil, 1 % of YE and a) 10 % of glucose (45 μg on a Chromatorod); b) 10 % of sucrose (95 μg on a Chromatorod).

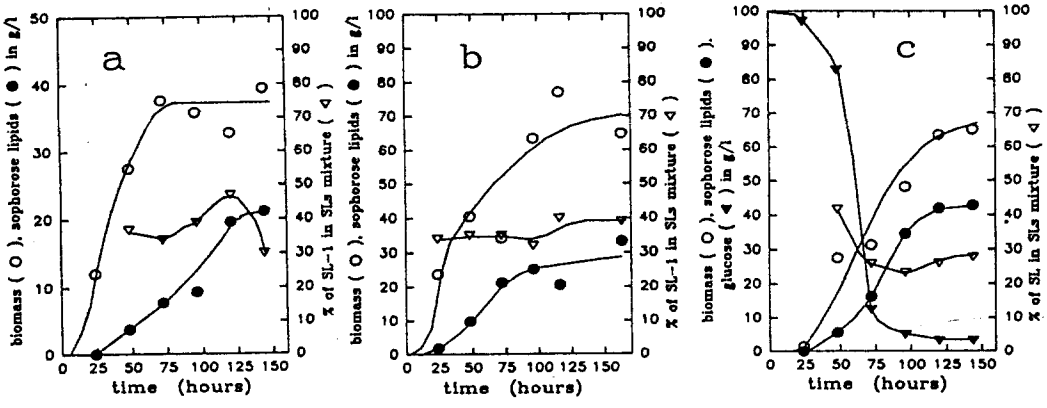

FIGURE 3 Cultivation of *Torulopsis bombicola* in Bellco jar fermenter on 10 % sunflower oil and a) 10 % sucrose with 0.25 % of yeast extract; b) 10 % sucrose with 1 % of yeast extract; c) 10 % glucose with 1 % of yeast extract.

TABLE I Effect of different concentration of yeast extract on biomass and sophorose lipids yields in shake flask cultivations (6 days) of *Torulopsis bombicola* on 9.5 % sunflower oil and 10 % glucose or sucrose.

	yeast extract (%)	biomass (g/l)	sophorose* lipids (g/l)	SL-1** % of mixture	other SLs** % of mixture
10 % glucose	0.25	9.2	38	26	74
	0.5	16.1	20	52	48
	1.0	13.3	16	76	23
	2.0	10.6	5	-	-
10 % sucrose	0.25	7.1	33	31	69
	0.5	11.1	9	50	50
	1.0	13.6	13	79	21
	2.0	18.0	17	79	21

* estimated by weight and corrected for oil content in isolates
**estimated by TLC/FID of isolated sophorose lipids

Considering the effect of varying YE levels, the yield of SL-1 was virtually constant (10 - 12 g/l) regardless of the type of sugar, but the yield of other SLs decreased with increasing YE levels.

Quite different relations were obtained when the cultivation was carried out in the fermenter and the culture was supplied with more air (2 vvm). Higher production (about 50%) was observed in the medium with 1 % of YE than with 0.25 % (Fig. 3ab). Comparing to the cultivation on glucose the production from sucrose was lower (Fig. 3bc). The composition of final mixtures of SLs was nearly the same in all cases and different of that isolated from flask experiments. The ratio between two groups of SLs changed during the cultivation but the extent of change was small. Cooper and Paddock (1984) concluded on the basis of TLC that the composition of mixture was not changing during the course of cultivation and our results are in this respect similar.

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