SHORT COMMUNICATIONS

Production of Sophorose Lipids by *Torulopsis bombicola* from Safflower Oil and Glucose

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The production of sophorose lipids increased with increasing concentrations of both safflower oil and glucose, and was profoundly influenced by the concentration of yeast extract. A high concentration of sophorose lipids (about 135 g/L) was obtained (in a 1-L Bellco stirred reactor) when the medium consisted of 10% glucose, 10.5% safflower oil, 0.1% urea, and 0.25–0.3% yeast extract. A similar yield of sophorose lipids also was obtained in a 20-L bioreactor. About 50% of the apolar sophorose lipid 1′,4″-lactone 6′,6″-diacetate (SL-1) was found in the mixture of sophorose lipids produced under these conditions.

KEY WORDS: Biosurfactant, fermentation, glycolipid, sophorose lipid, *Torulopsis*.

There has been increased interest in the production of sophorose lipids (SLs) by *Torulopsis* yeasts such as *T. apicola* (1), *T. bombicola* (2–6), *T. gropengiesseri* (7), and *T. petrophilum* (8). This interest stems from the ease of production, ability to act as surfactants, and biodegradability of SLs. In addition, cosmetics applications of SL derivatives also have been claimed (5). The production of about 120 g/L of SLs has been claimed with *T. bombicola* KSM-36 grown on high concentrations of glucose and palm oil (4,5), but exact conditions for such production were not reported. Investigations carried out on the optimization of SL production by *T. bombicola* ATCC 22214 (3,6) reported yields of about 75 g/L. Therefore, it would be valuable to study further the cultivation conditions for *T. bombicola* to improve the yield of SLs. Moreover, the composition of SLs produced under given conditions is important because it determines their industrial applicability. In this work, emphasis was placed on the study of optimum conditions for the cultivation of *T. bombicola* ATCC 22214 on safflower oil and glucose.

EXPERIMENTAL PROCEDURES

Microorganism and cultivation condition. Torulopsis bombicola ATCC 22214 was maintained on YM-agar slants at 4°C and transferred every month. The cultivations were carried out in a 1-L Bellco jar glass fermenter at 30°C with 0.7 L of a medium containing 0.1% urea, 0.5% $MgSO_4$, 1% KH_2PO_4, 0.1% NaCl, and varying amounts of glucose, safflower oil, and yeast extract (YE) that are specified later in the text. The culture was stirred at 450 rpm with an air flow rate of 1.2 L/min. The cultivation in the 20-L bioreactor (Chemap, Männedorf, Switzerland) was run with 15 L of a medium at 400 rpm and an air flow rate of 13.5 L/min. The medium (pH 4.5 ± 0.1) was sterilized with all components together at 121°C for 30 min.

Analysis. Biomass was determined after centrifuging of 5 mL of culture broth mixed with 10 mL of methanol/chloroform (10/L) for 20 min at 2000 *g*. The supernatant was discarded. The cells were washed with distilled water, dried at 105°C for 48 hr, and weighed.

Five mL of culture broth was mixed with 10 mL ethylacetate for determination of glucose concentration and glycolipid content. After centrifugation (20 min, 2000 *g*), the SLs in the ethyl acetate extract were determined by thin-layer chromatography/flame ionization detector (TLC/FID) (1 μL of sample was applied on a Chromatorod that was developed in chloroform/methanol (90/4) and scanned by Iatroscan TH-10). TLC/FID enables also the separation and estimation (9) of the least polar sophorose lipid (SL-1), which has been described as 17-L-([2′-O-β-D-glucopyranosyl-β-D-glucopyranosyl]-oxy)-octadecanoic acid 1,4′-lactone 6′,6″ diacetate [see Fig. 1, (6)]. The water layer was used for determination of glucose concentration by DNS method (10).

RESULTS AND DISCUSSION

The influence of medium composition of SL production was studied in order to find the optimal cultivation conditions. In our experiments, SL yield increased with increasing safflower oil concentration as well as with increasing glucose concentration (see Table 1). Thus, both carbon sources were needed in high concentration in order to obtain a high production of SLs. In this respect, our findings are in agreement with previous studies (3,5).

Besides the effect of oil and sugar concentrations, there was a profound effect of YE concentration of SL yield. As seen in Table 2, increasing YE concentration from 0.1% to 0.3% led to an increase in the yield of SLs. The maximum yield of SLs was obtained at 0.2–0.3% of YE. Above 0.3% of YE, the yields of SLs were much lower. This may be due to the fact that SLs have been reported to accumulate under nitrogen-limited conditions (3,6). Excess nitrogen-containing media presumably suppress SL production. Our results show that YE in low concentration enhanced SL production. However, low nitrogen concentration is not suitable for SL production because YE promotes growth and stimulates SL production. Studies carried out in shaken flasks with YE as a sole source of nitrogen have shown that 0.5% YE stimulated SL production compared with a medium with 0.1% YE (5). In our case, when urea was used besides YE as a nitrogen source, lower YE concentration was more favorable, suggesting that overall concentration of nitrogen in the medium is of importance. However, when the content of nitrogen in urea (47%) and in YE (about 10%, (11)) is compared, it becomes evident that 0.1% of urea actually substitutes for approximately 0.47% of YE and counts for about 2/3 of total nitrogen in the medium. Additional increase in urea concentration is not likely desirable as the production of SLs and growth of *T. bombicola* on sole urea have been found to be poor (3). Moreover, deficiency in vita-

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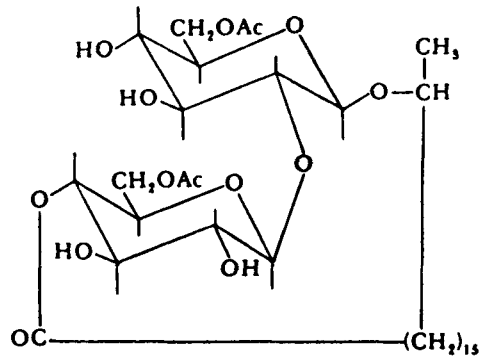


FIG. 1. Structure of SL-1.

TABLE 1

Effect of Glucose and Safflower Oil Concentration on the Yield of Sophorose Lipids Produced by *Torulopsis bombicola* in 1-L Fermenter[a]

Concentration oil		Yield of	
Safflower oil (%)	Glucose (%)	Biomass (g/L)	Sophorose lipids (g/L)
	2.5	22.27	17.51
10.5	5.0	30.71	36.2
	7.5	25.77	42.21
	10.0	28.39	44.5
2.5		20.10	15.42
5.0		29.07	34.2
7.5	10.0	22.85	37.1
10.0		24.78	44.5
10.5		25.12	46.78

[a]Medium with 0.1% yeast extract.

TABLE 2

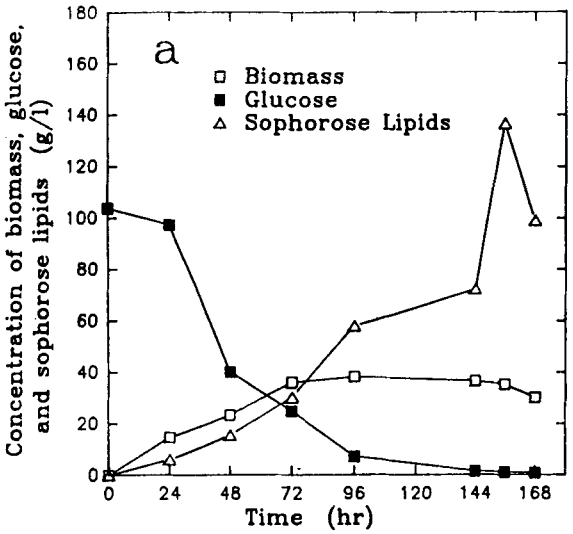
Effect of the Concentration of Yeast Extract on Sophorose Lipid and Biomass Yield and Composition of SL Produced by *T. bombicola* in 1-L Fermenter[a]

Concentration of yeast extract (%)	Concentration of		
	Biomass (g/L)	Sophorose lipids (g/L)	SL-1 in SL mixture (%)
0.1	24.5	46.8	35
0.2	34.1	106.5	27
0.3	35.2	136.6	50
0.4	44.8	45.8	31
0.5	48.2	10.2	65
1.0	58.3	2.6	61

[a]Medium with 10% glucose and 10.5% safflower oil.

mins like pantothenic acid, thiamin, and pyridoxin that are supplied by YE has been reported to decrease the amount of lipids synthetized by yeast (12).

The composition of the SL mixture as characterized here by the content of SL-1 was typically constant during a batch fermentation. Nevertheless, YE concentration influenced not only yield but also SL composition. It seems that higher YE concentration stimulated production of SL-1.



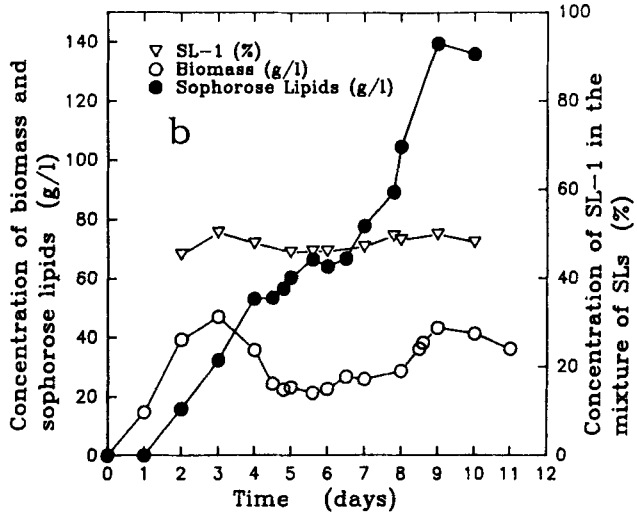


FIG. 2. The time course of the cultivation of *Torulopsis bombicola* in media containing 10% glucose, 10.5% safflower oil and a) 0.3% yeast extract in a 1-L fermenter; b) 0.25% yeast extract in a 20-L bioreactor.

Figure 2a shows the time course of cultivation in the 1-L fermenter under our optimum conditions. The concentration of biomass increased up to 72 hr, then remained constant and decreased slightly after 156 hr. Glucose was almost exhausted after 96 hr but SLs accumulated during both growth and stationary phases of the cultivation up to 158 hr.

To verify the high yields of SLs on a large scale, the cultivation was carried out in a 20-L bioreactor (Fig. 2b). The time course of the cultivation was similar to the cultivation in the 1-L fermenter although the maximum in SL concentration was reached after longer time. The SL-1 content in the mixture of SLs was about 50% under

these conditions. There is a clear possibility to influence the composition of SLs by medium composition but this will be connected to changes in the SL yield.

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