APPLIED AND ENVIRONMENTAL MICROBIOLOGY, June 1982, p. 1278–1283
0099-2240/82/061278-06$02.00/0
Vol. 43, No. 6

Sophorolipids from *Torulopsis bombicola*: Possible Relation to Alkane Uptake

SUSUMU ITO* AND SHIGEO INOUE

Tochigi Research Laboratories, Kao Soap Co. Ltd., Ichikaimachi, Hagagun, Tochigi 321-34, Japan

Received 6 August 1981/Accepted 21 February 1982

Torulopsis bombicola produces extracellular sophorolipids when it is grown on water-insoluble alkanes. Sophorolipids and related model compounds, which were not themselves used for growth, were found to stimulate markedly the growth of *T. bombicola* on alkanes. This stimulatory effect was restricted to growth on C_{10} to C_{20} alkanes, whereas no significant influence was observed for growth on fatty alcohols, fatty acids, glucose, or glycerol. The nonionic methyl ester of the glycolipid supported the greatest cell yield. However, a number of synthetic nonionic surfactants were unable to replace the glycolipid. When organisms were grown on hexadecane, stimulation of growth by sophorolipids was observed almost exclusively with strains of *Torulopsis* yeasts. In contrast, the growth of other typical alkane-utilizing yeasts, such as *Candida* and *Pichia* strains, was inhibited or not affected. It appears that sophorolipids are involved in alkane dissimilation by *T. bombicola* through an undetermined mechanism.

The production of extracellular glycolipids has been observed in yeast cultures (3, 16). However, the biological function of these lipids has not been fully investigated, and they have been considered to be secondary metabolites (1, 16).

The sophorolipids produced by *Torulopsis* sp. were first found by Spencer (18) in 1954. Tulloch et al. (19) identified the principal fractions of the glycolipid mixture from *Torulopsis apicola* (later classified as *Torulopsis bombicola* by Spencer et al. [15]) produced during fermentation of octadecane. The main components were an acidic sophoroside of 17-hydroxyoctadecanoic acid (type I) and its lactonic derivatives (types II and III), as shown in Fig. 1. We have shown previously that the type II lactone inhibits the growth of various alkane-utilizing yeasts on alkanes (9). To date, there have been no studies on the biological significance of the sophorolipids produced by *T. bombicola*.

This study was initiated to clarify the mechanism by which *Torulopsis* yeasts incorporate and metabolize water-insoluble alkanes. Our results suggest that sophorolipids are essential for the growth of these yeasts on water-insoluble alkanes.

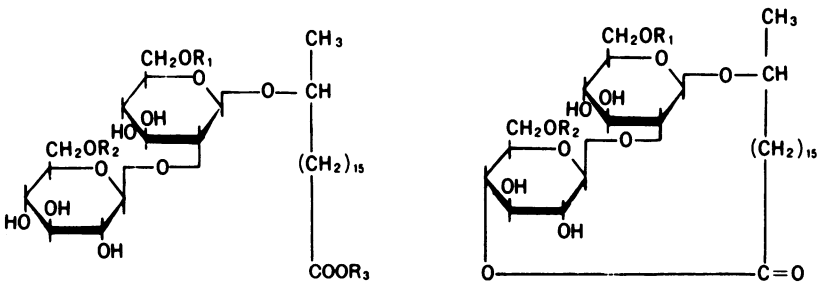
(Some of the results were presented at the 6th International Fermentation Symposium, London, Ontario, Canada, 1980.)

MATERIALS AND METHODS

Organisms. *T. bombicola* ATCC 22214 (the same strain used by Spencer et al. [15] and Tulloch et al. [19]) and *T. bombicola* KSM-36 were used extensively. The latter strain was isolated from a cabbage leaf as an alkane-utilizing yeast in our laboratory. This strain produced the same principal sophorolipid fractions that are produced by *T. bombicola* ATCC 22214 (9, 19) at concentrations ranging from 120 to 130 g/liter when it was grown in glucose broth amended with plant oils as sources of the fatty acid moieties of the glycolipids. The other yeasts used included 96 strains belonging to the following genera: *Torulopsis*, 40 strains belonging to 19 species; *Candida*, 20 strains belonging to 13 species; *Pichia*, 13 strains belonging to 11 species; *Cryptococcus*, 4 strains belonging to 3 species; *Metschnikowia*, 2 strains belonging to 2 species; *Debaryomyces*, 5 strains belonging to 5 species; *Lipomyces*, 2 strains belonging to 1 species; *Rhodotorula*, 4 strains belonging to 1 species; *Rhodosporidium*, 1 strain; *Lodderomyces*, 1 strain; *Saccharomycopsis*, 1 strain; *Schwaniomyces*, 2 strains belonging to 2 species; and *Endomyces*, 1 strain. The yeasts were maintained in YM slants at 4°C.

Media and cultivation. The medium in the YM slants contained the following (in grams per liter): yeast extract, 3.0; malt extract, 3.0; tryptone, 5.0; glucose, 10; and agar powder, 15. The pH of this medium was not adjusted.

The growth medium contained 7.5 g of yeast nitrogen base per liter, 2.0 g of yeast extract per liter, a concentration of a carbon source suitable for optimum growth, and distilled water (pH 5.6). The basal medium was this medium lacking the carbon source. Since omission of yeast extract from the basal medium resulted in variations in growth rate and cell yield, this nutrient was added to obtain reproducible results. Complete growth medium (100 ml) was placed in 500-ml Sakaguchi flasks or 10-ml portions were placed in test tubes (23 by 200 mm), and the preparations were autoclaved. Volatile alkanes were heated in sterile tubes in a boiling water bath for 10 min and added aseptically to the basal medium.



I : $R_1 = R_2 = Ac$, $R_3 = H$

Acid-SL : $R_1 = R_2 = R_3 = H$

Methyl-SL : $R_1 = R_2 = H$, $R_3 = Me$

II : $R_1 = R_2 = Ac$

III : $R_1 = H$, $R_2 = Ac$

FIG. 1. Structures of major components of sophorolipids produced by *T. bombicola*. Type I, 17-L-[(2'-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]octadecanoic acid 6',6''-diacetate; type II, 1,4''-lactone of type I; type III, 6'-deacetylated derivative of type II. As described in the text, the fatty acid moiety of the glycolipids from *T. bombicola* KSM-36 was replaced mainly by 17-hydroxyoctadecenoic acid. Ac, Acetyl; Me, methyl.

The inoculum size of a standardized yeast suspension from YM slants and the growth conditions were as follows. A 1-day-old slant culture was suspended in chilled saline and standardized to 0.5 to 0.6 units of absorbance at 650 nm (A_{650}). Flasks or test tubes containing growth medium were incubated aerobically at 30°C on a reciprocal shaker (125 strokes per min) or a test tube rotary shaker (300 rpm) with 0.2% standardized cell suspension. To study the interaction between sophorolipids (or synthetic surfactants) and the growth of a yeast, two sets of growth medium (one with the glycolipid and the other without the glycolipid) were incubated with the standardized cell suspension. The sophorolipids and synthetic surfactants used were sterilized separately in a boiling bath for 10 min.

Growth measurement. Cell growth was monitored by measuring the A_{650}, using appropriate dilutions made with chilled saline. As described previously (9), to remove insoluble carbon sources, which interfered with A_{650} readings, each culture flask was cooled to 4°C to solidify the substrate on the medium surface, and then ethyl acetate was placed onto the surface for several minutes. The solvent extraction did not result in erroneous A_{650} readings when the cooling pretreatment was included. Under the conditions described above, the lower limit of growth measurement was 0.05 to 0.1 A_{650} unit. Usually, control flasks produced no measurable A_{650} reading after 5 of 10 days of incubation. Growth was also measured in terms of dry cell mass. Cells from a known volume of culture medium were centrifuged at 18,000 rpm and washed twice with chilled saline. The resulting cell paste was dried at 80°C for several days and weighed. An A_{650} reading of 1.0 U corresponded to approximately 520 and 500 mg of dry cells per liter for *T. bombicola* KSM-36 and ATCC 22214, respectively.

Fermentation and isolation of sophorolipids. *T. bombicola* KSM-36 was used to obtain a crude sophorolipid mixture. The fermentation medium consisted of 10% (wt/vol) glucose, 0.5% (wt/vol) yeast extract, and 10% (wt/vol) safflower oil (pH 5.6). Safflower oil was added as a source of the fatty acid moiety of sophorolipid instead of octadecane. The organism was cultivated in 100-ml portions of the medium in Sakaguchi flasks at 30°C for 1 week on a reciprocal shaker.

Isolation of crude sophorolipids from the spent growth medium was performed by three successive extractions with 3 volumes of ethyl acetate. The solvent layer was evaporated to dryness in vacuo at 40°C. The resulting brownish paste was washed with hexane three times to remove the remaining safflower oil. The washed material was dried to yield 32 g of crude sophorolipid mixture (designated safflower-SL) from 1 liter of spent culture medium.

The structures of the main components of safflower-SL coincided basically with those reported by Tulloch et al. (19) and Ito et al. (9), as confirmed by ^1H- and ^{13}C-nuclear magnetic resonance spectrometry, infrared spectrophotometry, gas-liquid chromatography, and thin-layer chromatography. The fatty acid component of safflower-SL consisted mainly of 17-hydroxyoctadecenoic acid (92.2%), as revealed by gas chromatograph mass spectrometry. A ^{13}C-magnetic resonance spectrum of safflower-SL in C^2HCl_3 also indicated olefinic carbon signals at 129.8 and 130.3 ppm from an internal standard, $(CH_3)_4Si$.

Syntheses of related model sophorolipid compounds. The related model sophorolipid compound 17-L [(2'-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]octadecenoic acid (acid-SL; $R_1 = R_2 = R_3 = H$) and its methyl ester (methyl-SL; $R_1 = R_2 = H$; R_3 = methyl) were synthesized and purified by the method of Tulloch et al. (19). Acid-SL was synthesized by alkaline hydrolysis of safflower-SL (10 g) under reflux with 100 ml of a 1.0 N ethanolic KOH solution at 80°C for 30 min. Methyl-SL was prepared from safflower-SL (10 g) by reflux with 100 ml of 0.1 N methanolic sodium methoxide for 30 min. Methyl-SL-3',4',6',2'',3'',4'',6''-heptaacetate was synthesized from methyl-SL by the conventional method (19) and was purified by silica gel column chromatography. For acid-SL, the maximum absorption peaks of the infrared spectra in KBr were as follows: 1,700 cm^{-1}, indicating carboxyl group; and absent at 1,740 and 1,380 cm^{-1}, indicating no acetyl

group. For methyl-SL, the peaks were as follows: 1,740 and 1,440 cm^{-1}, indicating methyl ester; and absent at 1,380 cm^{-1}, indicating no acetyl group. Other physicochemical properties of the model compounds were similar to those reported by Tulloch et al. (19).

Chemicals. Unless otherwise specified, the alkanes and other reagents used were from Wako Pure Chemicals, Osaka, Japan, and were certified reagent grade. Yeast nitrogen base was obtained from Difco Laboratories, Detroit, Mich., yeast extract was from Daigo Eiyo, Osaka, Japan, α-sophorose was from Senn Chemicals, Dielsdorf, Switzerland, and $(CH_3)_4Si$ and C^2HCl_3 were purchased from E. Merck AG, Darmstadt, Germany. A total of 43 synthetic nonionic surfactants with hydrophile-lipophile balance values ranging from 3.6 to 19.5 were obtained from Kao Soap Co. Ltd., Tokyo, Japan, and were used without further purification. A sophorolipid product of *Candida bogoriensis*, 13-[(2′-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]docosanoic acid (candidal SL) (5), was kindly supplied by R. J. Light, Florida State University, Tallahassee.

RESULTS

Growth responses on various carbon sources. The growth responses of *T. bombicola* ATCC 22214 and KSM-36 to a number of carbon sources were examined (Table 1). Short-chain alkanes (C_5 to C_{10}) and long-chain alkanes (C_{14} to C_{22}) were not able to support growth of these yeast strains, whereas C_{10} to C_{13} alkanes, fatty alcohols, fatty acids, glucose, and glycerol supported growth. Toxicity of the alkanes was eliminated by supplementing glucose cultures with 2% alkane, which had no effect on the growth of the yeasts.

Stimulation of growth by sophorolipids. Supplementation of the basal medium containing hexadecane with safflower-SL or its derivatives allowed the growth of *T. bombicola* KSM-36 (Fig. 2). Synthetic methyl-SL supported the greatest cell yield after 6 days of incubation. The maximum cell yield with safflower-SL was similar, although the growth rate was reduced slightly. The growth stimulation by acid-SL was less than the stimulation observed with methyl-SL or safflower-SL, and the growth seemed to reach a plateau after 8 days. Candidal-SL from *C. bogoriensis* (5) did not serve as an alternate growth stimulator. The growth of a control culture (no sophorolipid) lagged for more than 8 days, and the stationary phase was not reached until after 14 to 20 days (data not shown). The final cell yield of the control culture was similar to the yields obtained from cultures containing hexadecane and sophorolipids. The amount of growth on basal medium alone was negligible.

The possibility existed that sophorolipid degradation preceded hexadecane utilization and the resulting products stimulated growth of the organism. This possibility was evaluated by growing the yeasts with hexadecane and 16-hydroxypalmitic acid (one of the fatty acid moieties of sophorolipid [19]). After 8 days of incubation, no detectable growth resulted from these additives. In addition, when sophorose or methyl-SL heptaacetate was added, no growth occurred on the basal medium containing hexadecane. The same growth responses were observed with *T. bombicola* ATCC 22214. Thus, *T. bombicola* KSM-36 was used for all further experiments.

TABLE 1. Growth responses of *T. bombicola* on various carbon sources

Carbon source added	Concn (%)	Growth (A_{650} units) after 7 days[a]	
		Strain KSM-36	Strain ATCC 22214
Pentane	2	0.0	0.0
Hexane	2	0.0	0.0
Heptane	2	0.0	0.0
Octane	2	0.0	0.0
Nonane	2	0.0	0.0
Decane	2	3.2	3.3
Undecane	2	3.5	3.2
Dodecane	2	8.2	7.9
Tridecane	2	1.4	0.9
Tetradecane	2	0.0	0.0
Pentadecane	2	0.0	0.0
Hexadecane	2	0.0	0.0
Octadecane	2	0.0	0.0
Eicosane	2	0.0	0.0
Docosane	2	0.0	0.0
Dodecan-1-ol	2	4.3	3.9
Tetradecan-1-ol	2	8.2	8.4
Hexadecan-1-ol	2	9.2	9.1
Methyl dodecanoate	2	2.2[b]	2.5[b]
Methyl tetradecanoate	2	4.2[b]	4.4[b]
Methyl hexadecanoate	2	6.2[b]	6.0[b]
Methyl octadecanoate	0.5	2.2[b]	2.3[b]
Hexadecanoic acid	1	4.2	3.8
16-Hydroxyhexadecanoic acid	1	5.8	6.2
Glucose	1	7.2[c]	7.0[c]
Glycerol	2	3.9[b]	3.8[b]

[a] Test tube cultures. The amounts of growth were determined by measuring the absorbance of broth diluted 10-fold.
[b] Cultured for 2 days.
[c] Cultured for 1 day.

Effect of methyl-SL. In the presence of 0.02% methyl-SL, the growth of *T.bombicola* KSM-36 was measured on the basal medium containing various carbon sources (Table 2). The amount of growth of strain KSM-36 on C_{10} to C_{20} alkanes was consistently greater in the presence of methyl-SL. In contrast, growth on C_5 to C_9 and C_{22} alkanes was not affected by methyl-SL. Table 3 shows that methyl-SL had no significant influence on the growth with other carbon sources,

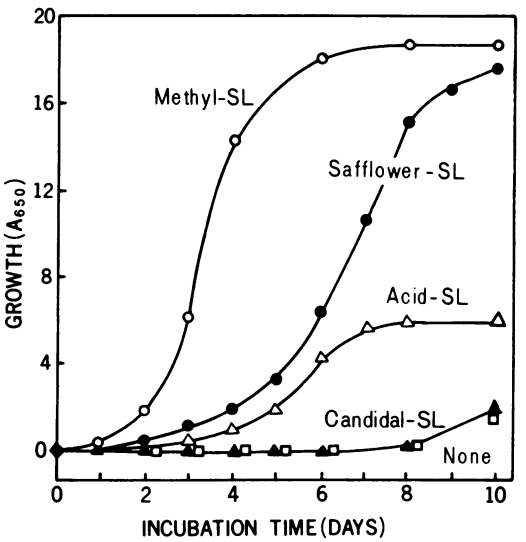


FIG. 2. Stimulation of growth of *T. bombicola* KSM-36 on hexadecane by sophorolipids. Symbols: supplementation with 0.04% safflower-SL (●), 0.02% acid-SL (△), 0.02% methyl-SL (○), or 0.02% candidal-SL (▲) or no addition (□).

such as fatty alcohols, fatty acids, glucose, and glycerol. Since methyl-SL can be classified structurally as a nonionic surfactant, it was of interest to examine the effects of synthetic nonionic surfactants on the growth of *T. bombicola* on water-insoluble alkanes. None of the tested synthetic nonionic surfactants with hydrophile-lipophile balance values ranging from 3.6 to 19.5 either stimulated or inhibited the growth of *T. bombicola* KSM-36 on hexadecane (data not shown). In addition, the growth yields obtained with glucose were not affected by the synthetic surfactants.

TABLE 2. Growth of *T. bombicola* KSM-36 on individual alkanes in the presence of methyl-SL

Alkane added[a]	Growth (A_{650} units) after incubation for[a]:		
	4 Days	7 Days	10 Days
Pentane	0.0/0.0[b]	0.0/0.0	0.0/0.0
Hexane	0.0/0.0	0.0/0.0	0.0/0.0
Heptane	0.0/0.0	0.0/0.0	0.0/0.0
Octane	0.0/0.0	0.0/0.0	0.0/0.0
Nonane	0.0/0.0	0.0/0.0	0.0/0.0
Decane	3.0/1.8	7.2/5.0	7.9/6.3
Undecane	3.0/2.4	11.9/6.2	14.3/8.4
Dodecane	7.7/5.9	17.3/12.4	19.9/14.5
Tridecane	6.6/0.7	12.8/3.9	14.9/11.2
Tetradecane	7.5/0.0	18.3/0.0	20.0/2.0
Pentadecane	2.8/0.0	18.8/0.0	20.1/1.0
Hexadecane	8.1/0.0	19.0/0.0	20.3/2.3
Octadecane	3.5/0.0	9.9/0.0	14.1/1.3
Eicosane	0.9/0.0	1.5/0.0	2.0/0.0
Docosane	0.0/0.0	0.0/0.0	0.0/0.0

[a] Flask cultures. Methyl-SL was added at a concentration of 0.02%; each alkane was added at a concentration of 2.0%. Values are the means of duplicate flasks.

[b] Amount of growth with methyl-SL/amount of growth without methyl-SL.

TABLE 3. Effect of methyl-SL on the growth of *T. bombicola* KSM-36 with various carbon sources

Carbon source	Concn (%)	Growth (A_{650} units) after 5 days[a]	
		No addition	0.02% Methyl-SL added
Tetradecan-1-ol	2.0	7.5	9.4
Hexadecan-1-ol	2.0	10.1	12.5
Tetradecanoic acid	1.0	2.5	3.2
Hexadecanoic acid	1.0	4.5	5.3
Methyl tetradecanoate	2.0	2.6[b]	2.7[b]
Methyl hexadecanoate	2.0	6.2[b]	6.0[b]
Glucose	1.0	6.3[c]	6.2[c]
Glycerol	2.0	3.6[b]	3.7[b]
Hexadecane	2.0	0.0[b]	4.7[b]
		0.0	15.5

[a] Values are the means of duplicate flasks.
[b] Cultured for 2 days.
[c] Cultured for 1 day.

Growth of various yeasts with sophorolipids. The growth responses on safflower-SL and methyl-SL were examined for 98 yeast strains representing 13 genera on hexadecane (Table 4). Growth stimulation by safflower-SL or methyl-SL or both was observed almost exclusively with *Torulopsis* yeasts. In contrast, the growth of typical alkane-utilizing yeasts belonging to the genera *Candida, Pichia, Debaryomyces, Saccharomycopsis, Endomyces*, and *Lodderomyces* was inhibited or not affected.

DISCUSSION

The first step in alkane fermentation by microorganisms is the uptake of exogenous alkanes by the cells. Microorganisms that grow on petroleum or alkanes as the sole source of carbon often produce surfactant-like lipids, such as trehalose lipid (17), peptide lipid (8), corynemycolic acid (2), rhamnolipids (7, 10, 11), and a highly acidic polysaccharide (20). These compounds lower the surface tension of the growth medium and may emulsify the growth substrates (4). Hisatsuka et al. (7) studied the rhamnolipid from *Pseudomonas aeruginosa* S_7B_1, which stimulated the growth of this strain on alkanes. A requirement for two kinds of rhamnolipids in alkane fermentation was also reported by Itoh et al. (10, 11), who used the rhamnolipid-less mutants of *P. aeruginosa* KY 4025. Such results

TABLE 4. Growth of various yeasts on hexadecane in the presence of sophorolipids

Yeast tested	Growth (g of dry cells per liter)[a]		
	No addition	0.04% Safflower-SL	0.02% Methyl-SL
T. bombicola KSM-36	0.05	1.96	5.05 (5)
T. bombicola ATCC 22214	0.04	1.98	4.60 (5)
T. bombicola PRL 322-73	0.92	9.70	4.96 (5)
T. bombicola NRRL Y-5391	0.92	4.89	5.31 (5)
T. apicola PRL 123-64	0.34	1.94	2.74 (5)
T. apicola IFO 1039	0.30	3.26	0.30 (5)
Torulopsis gropengiesseri IFO 0659	0.11	6.22	3.54 (5)
T. gropengiesseri NRRL 1445N	0.06	2.62	3.37 (5)
T. gropengiesseri CBS 156	1.11	5.93	2.67 (5)
Torulopsis magnoliae IFO 1230	0.98	ND[b]	2.25 (3)
T. magnoliae IFO 0661	2.16	2.82	3.11 (5)
T. magnoliae ATCC 12573	3.96	ND	4.51 (2)
Torulopsis candida AHU 4121	1.91	ND	5.32 (4)
Candida lipolytica IAM 4964	7.94	1.03	0.32 (2)
Candida tropicalis IAM 4185	9.93	0.02	0.18 (2)
Candida parapsilosis IFO 1022	6.04	0.89	ND (2)
Pichia farinosa IFO 1163	4.49	10.32	3.17 (2)
Pichia polymorpha IFO 1166	3.60	0.53	2.54 (2)
Pichia guilliermondii CBS 2030	4.57	0.01	1.30 (2)
Pichia stipitis CBS 5773	3.56	0.01	1.30 (2)
Pichia media CBS 5521	1.93	0.04	0.14 (5)
Debaryomyces vanriji IFO 0934	8.44	0.81	0.96 (3)
Saccharomycopsis lipolytica CBS 6124	5.78	ND	3.52 (2)
Endomyces oventensis CBS 192-55	2.93	0.67	0.87 (6)
Lodderomyces elongisporus CBS 2605	2.39	0.65	1.79 (6)

[a] Test tube cultures. The numbers in parentheses indicate the number of days of incubation.
[b] ND, Not determined.

suggest that lipids having surfactant-like structures play an important role in the uptake of alkanes and that the function of the lipids can be attributed to their emulsifying activities in the oil-water phase.

Our results indicate that safflower-SL stimulates the growth of *T. bombicola* and some species of *Torulopsis* on water-insoluble alkanes. The active components in safflower-SL from *T. bombicola* KSM-36 are still obscure in that the glycolipid mixture seems to be structurally more complicated than the mixtures reported by Tulloch et al. (19) for *T. bombicola* ATCC 22214. The type II and III lactones purified from safflower-SL are not responsible for the growth stimulation (unpublished data). The former lactone inhibits the growth of typical alkane-utilizing yeasts, such as *Candida*, on alkanes at low concentrations (9). Safflower-SL and its derivatives have structurally surfactant-like properties and stimulate specifically the growth of *T. bombicola* on alkanes. Synthetic nonionic surfactants cannot replace sophorolipid. These results indicate indirectly that if sophorolipid acts as a surface-active agent, the basal structure of the glycolipid appears to be a plausible candidate for the growth stimulant of the yeast on alkanes.

In addition, one important characteristic in alkane uptake by yeasts is that cell walls should acquire a more lipophilic character after cultivation on alkanes, facilitating contact with alkanes (14). Käppeli and colleagues (12, 13) have recently isolated a surface-localized polysaccharide-fatty acid complex from alkane-grown *Candida tropicalis* responsible for the nonenzymatic contact with alkanes. Although insufficient evidence is available to explain the function of sophorolipid in alkane fermentation by *T. bombicola*, it is noteworthy that the cell wall of this yeast has no binding affinity for alkanes during growth in the presence of sophorolipids. In addition, safflower-SL and its derivatives do not have emulsifying activities for alkanes (unpublished data). These results suggest involvement of an unidentified mechanism for uptake of water-insoluble alkanes by *T. bombicola*. The alkane uptake model characteristic of this yeast is currently under study.

A peptide lipid has been found in the broth of *Candida petrophilum* cultures, and the growth of this yeast on an alkane was stimulated slightly by adding broth containing the peptide lipid (8). However, it is not clear that the peptide lipid itself functions as a stimulant in alkane fermentation. Therefore, no alkane-utilizing yeast has been reported to require a specific biosurfactant for growth on alkanes. *T. bombicola* seems to be the first such yeast found.

ACKNOWLEDGMENTS

We thank K. Komagata of the University of Tokyo for his continuous encouragement during this study and S. Takao of Hokkaido University for a generous supply of many strains of alkane-utilizing yeasts. The expert technical work of Y. Akiyama is gratefully acknowledged. We are indebted to M. Kinta for assistance with the early phases of this investigation.

LITERATURE CITED

1. **Bentley, R., and I. M. Campbell.** 1968. Secondary metabolism of fungi. Comp. Biochem. Physiol. **20**:415–489.
2. **Cooper, D. G., J. E. Zajic, and D. F. Gerson.** 1979. Production of surface-active lipids by *Corynebacterium lepus*. Appl. Environ. Microbiol. **37**:4–10.
3. **di Menna, M. E.** 1958. Two new species of yeasts from New Zealand. J. Gen. Microbiol. **18**:269–272.
4. **Erickson, L. E., and T. Nakahara.** 1975. Growth in cultures with two liquid phases: hydrocarbon uptake and transport. Process Biochem. **10**:9–13.
5. **Esders, T. W., and R. J. Light.** 1972. Glucosyl- and acetyltransferases in the biosynthesis of glycolipids from *Candida bogoriensis*. J. Biol. Chem. **247**:1375–1386.
6. **Heinz, A., A. P. Tulluch, and J. F. T. Spencer.** 1969 Stereospecific hydroxylation of long chain compounds by a species of *Torulopsis*. J. Biol. Chem. **244**:882–888.
7. **Hisatsuka, K., T. Nakahara, N. Sano, and K. Yamada.** 1971. Formation of rhamnolipid by *Pseudomonas aeruginosa* and its function in hydrocarbon fermentation. Agric. Biol. Chem. **35**:686–692.
8. **Iguchi, T., I. Takeda, and H. Osawa.** 1969. Emulsifying factor of hydrocarbon produced by a hydrocarbon-assimilating yeast. Agric. Biol. Chem. **33**:1657–1658.
9. **Ito, S., M. Kinta, and S. Inoue.** 1980. Growth of yeasts on *n*-alkanes: inhibition by a lactonic sophorolipid produced by *Torulopsis bombicola*. Agric. Biol. Chem. **44**:2221–2223.
10. **Itoh, S., H. Honda, F. Tomita, and T. Suzuki.** 1971. Rhamnolipids produced by *Pseudomonas aeruginosa* grown on *n*-paraffin (mixture of C_{12}, C_{13} and C_{14} fractions). J. Antibiot. **29**:855–859.
11. **Itoh, S., and T. Suzuki.** 1972. Effect of rhamnolipids on growth of *Pseudomonas aeruginosa* mutant deficient in *n*-paraffin utilizing ability. Agric. Biol. Chem., **36**:2233–2235.
12. **Käppeli, O., and A. Fiechter.** 1977. Component from the cell surface of the hydrocarbon-utilizing yeast *Candida tropicalis* with possible relation to hydrocarbon transport. J. Bacteriol. **131**:917–921.
13. **Käppeli, O., M. Müller, and A. Fiechter.** 1978. Chemical and structural alterations at the cell surface of *Candida tropicalis*, induced by hydrocarbon substrate. J. Bacteriol. **133**:952–958.
14. **Mimura, A., S. Watanabe, and I. Takeda.** 1971. Biochemical engineering analysis of hydrocarbon fermentation. III. Analysis of emulsification phenomena. J. Ferment. Technol. **49**:255–271.
15. **Spencer, J. F. T., P. A. J. Gorin, and A. P. Tulloch.** 1970. *Torulopsis bombicola* sp. n. Antonie van Leeuwenhoek J. Microbiol. Serol. **36**:129–133.
16. **Stodola, F. H., M. H. Deinema, and J. F. T. Spencer.** 1967. Extracellular lipids of yeasts. Bacteriol. Rev. **31**:194–213.
17. **Suzuki, T., K. Tanaka, T. Matsubara, and S. Kinoshita.** 1969. Trehalose lipid and α-branched-β-hydroxy fatty acid formed by bacteria grown on *n*-alkanes. Agric. Biol. Chem. **33**:1619–1627.
18. **Tulloch, A. P.** 1976. Structures of extracellular glycolipids produced by yeasts, p. 329–345. *In* L. A. Witting (ed.), Glycolipid methodology. American Oil Chemists' Society, Champaign, Ill.
19. **Tulloch, A. P., A. Hill, and J. F. T. Spencer.** 1968. Structure and reactions of lactonic and acidic sophorosides of 17-hydroxyoctadecanoic acid. Can. J. Chem. **46**:3337–3351.
20. **Zuckerberg, A., A. Diver, Z. Peeri, D. L. Gutnick, and E. Rosenberg.** 1979. Emulsifier of *Arthrobacter* RAG-1: chemical and physical properties. Appl. Environ. Microbiol. **37**:414–420.