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Sophorolipid Production by *Candida bombicola* ATCC 22214 from a Corn-Oil Processing Byproduct

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Abstract Sophorolipid was produced by *Candida bombicola* ATCC 22214 from soybean dark oil, a byproduct of soybean oil processing. With a fed-batch culture of *C. bombicola* for 7 days, 90 g/l of sophorolipid was obtained. The CMC (critical micelle concentration) and minimum surface tension of the sophorolipid in aqueous solution were found to be 150 mg/l and 48 mN/m, respectively. The dispersion capability of sophorolipid was higher than that of the chemical surfactants such as SDS and Brij30. The molar solubility ratio (MSR) of 4-methylnaphthalene was 0.2. Linoleic and oleic acids were the main constituents of the fatty acid composition of the sophorolipid. The sophorolipid showed antimicrobial activity against *Propionibacterium acne* and *Bacillus subtilis*.

Key words: Biosurfactant, sophorolipid, dark oil, *Candida bombicola*, antimicrobial activity

Worldwide markets for surfactants are increasing annually, however, almost all the commercially available surfactants are chemically derived from petroleum. Consequently, interest in biosurfactant has been steadily increasing in recent years, due to their potential advantages over the chemical surfactants, such as lower toxicity, higher biodegradability, better environmental compatibility, and higher selectivity and higher specific activity at extreme temperatures, pH, and salinity [1, 13, 15, 18].

Sophorolipid, a glycolipid-type biosurfactant, has an 8–10 hydrophilic-lipophilic balance (HLB) value. Several applications have been proposed in diverse fields such as cosmetics, pharmaceuticals [10], enhanced oil recovery [12], and harmful algal bloom [2].

For the successful commercialization of biosurfactant, they must compete with synthetic surfactants at all levels. The main economic factors in biosurfactant production from microorganisms are the costs of raw materials, separation, and their productivity. Since half of the raw materials are directly transformed to biosurfactant, the cost of raw materials makes up approximately 40–50 percent of the whole expense for biosurfactant production. Therefore, the selection and development of cheaper raw materials are essential in reducing the entire production costs. Many research studies on sophorolipid production used diverse raw materials such as carbohydrates [11], vegetable oils [17, 19], animal fats [6], n-alkanes [5], and single cell oils [4].

A byproduct from vegetable oil processing, the so-called 'soybean dark oil (SDO),' has a black color and is an inedible oil. Although it consists of 65–75% fatty acid and 20–30% triglyceride, most of SDO have been discarded, and only a small fraction has been recycled for the production of industrial-grade fatty acids. Since the fatty acid profiles of SDO are similar to vegetable oils such as soybean oil or corn oil, we examined SDO's potential use as a raw material for the production of sophorolipid. In this study, we evaluated soybean dark oil (SDO) and its characteristics as a potential candidate for cheap raw material to produce sophorolipid by *C. bombicola*.

Materials and Methods

Strains and Culture

The yeast, *Candida bombicola* ATCC 22214, was maintained on YM agar slants with monthly transfers. The standard medium for cultivation contained (per liter distilled water) glucose 100 g, yeast extract 5 g, KH_2PO_4 1 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, $CaCl_2 \cdot 2H_2O$ 0.1 g, NaCl 0.1 g, peptone 0.7 g, and 10% (w/v) of SDO, corn oil, or soybean oil.

Five percent (v/v) of culture suspension was used as the inoculums for a 2.5-l jar fermenter (Kobiotech, Korea). Conditions for fermenter operation were: working volume, 1 l; temperature, 30°C; pH, 3.5; agitation, 550 rpm; aeration rate, 1 VVM; and culture time, 7 days.

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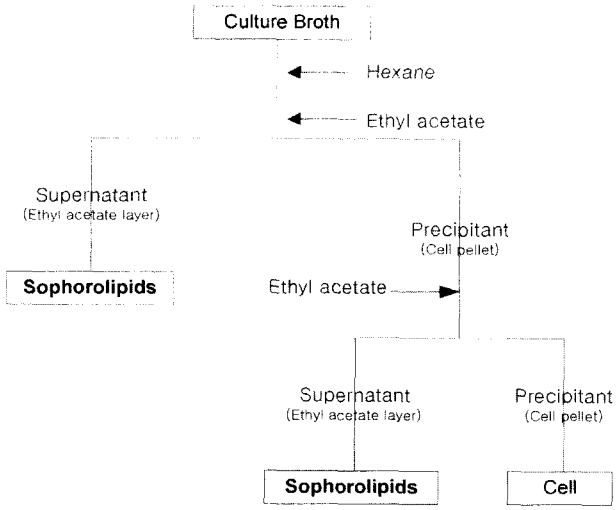

Fig. 1. Schematic diagram of sophorolipid preparation.

Sophorolipid Separation

The culture broth was extracted three times with the same volume of ethyl acetate. To obtain crude sophorolipid, the ethyl acetate layer was evaporated with a rotary vacuum evaporator (Eyela, Tokyo Rikakikai, Japan), and residual oil was removed by extracting with hexane (Fig. 1).

Surface Tension, CMC, and Dispersion Power Measurement

The surface tensions of each candidate's supernatant were measured with a du Nuoy tensiometer (Surface Tensiomat21, Fisher Scientific, Pittsburgh, PA, U.S.A.), using a platinum ring. Critical micelle concentration (CMC) is the concentration at which the micelle begins to form. CMC corresponds to the point where the surface tension is minimum and was determined with the same tension meter at 25°C. For dispersion power determination, 0.3 g of Fe_2O_3 powder in 100 ml distilled water was stirred vigorously with various concentrations of sophorolipid, and the suspension was poured into a 100-ml mass cyclinder. After 10 min, 2 ml of the sample from the top 20 ml were diluted with 25 ml of distilled water. Absorbance at 640 nm was measured. All the measurements were the average of two samples.

Solubility of Hydrocarbon in Aqueous Solution

Aqueous solubility experiments were performed in a 20-ml vial using 10 ml of several concentrations of sophorolipid in the presence of excess amounts of 4-methylnaphthalene. The vials were equilibrated for 48 h, and the samples (2 ml) were collected and extracted with 4 ml of hexane. The extracts were analyzed for 4-methylnaphthalene using gas chromatography equipped with FID (GC-14B, Shimadzu, Japan). The molar solubility ratio (MSR) was expressed as the molar concentration of the hydrocarbon (2-methylnaphthalene) solubilized in the aqueous solution containing the sophorolipid.

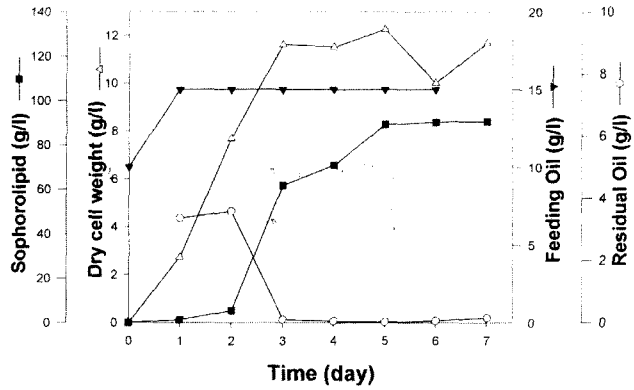

Fig. 2. Time course of sophorolipid production by *C. bombicola*. Medium: 10% initial glucose, feeding 15 g soybean dark oil/day.

Types and Fatty Acid Compositions in the Sophorolipid

To analyze the fatty acid profile of the sophorolipid and oils, 1 g of each sample was dissolved in 15 ml of 0.5 N KOH/methanol and reacted in a 100°C sand bath for 3 h for cleavage of the ester bond. Then, 20 ml of BF_3/methanol were added and incubated at 90°C for 15 min to form fatty acid methyl ester. The formed methyl ester was extracted using with petroleum containing ether and hexane, and the extract was analyzed by a GC/FID equipped with a Supelco Wax 10 (30 m×0.53 mm×1 μm) column.

Antimicrobial Activity

For the antimicrobial activity test, several bacterial strains were obtained from Korea Culture Type Collection (KCTC). For *Propionibacterium acne* cultivation, LAB M reinforced clostridial medium was used [7], and LB medium was used for other bacterial strains. Inhibitory concentration (IC) was defined as the concentration at which the number of colonies began to decrease. *P. acne* was incubated for 6-7 days under anaerobic conditions in an anaerobic jar at 37°C, whereas other bacteria were incubated for only 1-2 days.

Results and Discussion

Sophorolipid Production

Sophorolipid production by yeast was strongly affected by the use of different lipidic substrates as the second C-source [8]. Because the price of SDO is less than half the

Table 1. Effects of oils on cell growth and sophorolipid production.

	Concentration (g/l)		
	Sophorolipid	Biomass	Residual glucose
SDO*	90	12.3	4
Corn oil	100	7.6	2
Soybean oil	24	7.9	6

*SDO: Soybean dark oil.

Table 2. Fatty acids composition of oils and sophorolipids.

Oils and sophorolipids	Fatty acid (%)				
	C16:0	C18:0	C18:1	C18:2	C18:3
Corn oil	9.75	1.65	24.65	61.95	0.59
Sophorolipid produced	7.83	0.67	27.41	56.50	2.23
Soybean oil	10.30	3.6	21.91	56.13	6.22
Sophorolipid produced	8.27	2.19	26.45	60.88	0.51
Soybean dark oil	6.50	2.63	16.10	65.41	5.78
Sophorolipid produced	9.20	4.26	22.13	51.10	4.87

price of other oils, it is prudent to determine the productivity and the characterization of sophorolipid produced from SDO.

Figure 2 shows the variations in biomass, sophorolipid production, and amounts of the second substrates in the fed-batch cultivation with an initial glucose concentration of 100 g/l and 100 g/l SDO as the second substrate. Sophorolipid production began in the late exponential phase, and the increased formation was observed upon the exhaustion of the second substrate in the stationary phase. Because biomass variation was not observed in this phase, we assumed that most of the added second substrate and glucose were transformed to sophorolipid.

Biomass, sophorolipid production, and residual glucose were compared to clarify the effect of the second substrate (Table 1). Corn oil showed the highest level of sophorolipid production (100 g/l). When SDO was used, 90 g/l of sophorolipid was produced. The amount is similar to 90–110 g/l from lactose and canola oil [19].

Characterization of Sophorolipid

Two types of sophorolipid were produced from SDO at room temperature and atmospheric pressure; lactone-type and acid-type. The ratio of the two types was determined by FT-IR and TLC/FID. The types of sophorolipid from corn oil and soybean oil were mainly the acid type, comprising 85.49% and 96.06%, respectively. On the contrary, the lactone type was the major form of sophorolipid from SDO. Lactone-type sophorolipid has been used in cosmetics as antidandruff treatments, bacteriostatic agents, deodorants [14], and as agents for stimulating skin fibroblast metabolism [3]. The acidic sophorolipid has been found to be therapeutically active for skin treatment, particularly as agents for fibrinolysis, healing, desquamation, depigmenting, and macrophage activation [16].

Table 3. CMC, MSR, and K_{mw} values of sophorolipids produced from various oils.

Oils	Surface tension (mN/m)	CMC (mg/l)	MSR
Soybean dark oil	48	150	0.2
Corn oil	41	82	0.2
Soybean oil	40.5	88	0.5

CMC (Critical Micelle Concentration). MSR (Molar Solubility Ratio of 2-methyl naphthalene).

Table 4. Dispersion power of sophorolipids.

		Dispersion power (OD 640 nm)	
		2 h	24 h
	Water	0.12	0.02
Sophorolipid (raw material)	(Corn oil)	0.64	0.13
	(SDO*)	0.39	0.09
	(Soybean oil)	0.92	0.25
Chemical surfactant	Tween 80	1.93	0.71
	SDS	0.20	0.07
	Brij 30	0.66	0.10

*SDO: soybean dark oil.

Fatty acid compositions were compared between the sophorolipid and the secondary substrates (Table 2). The fatty acid compositions of the substrates were mainly oleic acid and linolic acid, and were similar profiles to those of sophorolipids produced. Also, the fatty acid composition of sophorolipid from other substrates showed a similar trend. This result indicates that SDO could replace second substrates such as vegetative oils without any significant change of composition.

Surface-Active Property of Sophorolipid

Further experiments were carried out to evaluate the sophorolipids potential to function as a surfactant (Table 3). CMC and the minimum surface tension of the sophorolipid produced from SDO in aqueous solution were 150 mg/l and 48.0 mN/m, respectively, whereas those values from corn oil were 82 mg/l and 41.0 mN/m, and from soybean oil were present at 88 mg/l and 40.5 mN/m, respectively. The MSR and $\log K_{mw}$, which represent the solubilizing power of hydrocarbon in the surfactant solution, were 0.21 and 3.56 respectively, and these values were similar to those of other sophorolipids produced from different substrates (Table 3).

The dispersion power of the sophorolipid produced from vegetable oils was higher than the chemical surfactants SDS and Brij 30. Although the sophorolipid from SDO showed weaker dispersion power than other sophorolipids and chemical surfactants except SDS at 2 h, the activity at 24 h showed values similar to those of SDS and Brij 30 (Table 4).

Table 5. Antimicrobial effect of sophorolipid on various microorganisms.

Microorganisms	IC (mg/l)
Bacillis subtilis	4
Propionibacterium acne	0.5
Escherichia coli	10,000

IC: Inhibitory Concentration.

Table 6. IC (inhibitory concentration) of sophorolipids against *Propionibacterium acne.*

Oil source for sophorolipid production	Corn oil	Soybean dark oil	Soybean oil	Canola oil	Irgasan DP-300*
IC (mg/l)	<1.5	<1.5	<16	<0.5	<3

*Irgasan DP-300: a commercial agent.

Antimicrobial Activity

The inhibitory concentrations (IC) of the sophorolipid against various microorganisms are shown in Table 5. The IC was 4.0 and 0.5 mg/l against *Bacillus subtilus* and *Propionibacterium acne*, respectively. Interestingly, the inhibition of the *E. coli* cell growth was observed at higher concentration (10,000 ppm). These results are consistent with those of sophorolipid produced from canola oil [9].

The sophorolipid showed antimicrobial activity toward *P. acne*, which is an acne-causing bacterium (Table 6), and the IC of the sophorolipid was comparable with a commercial antimicrobial agent. The IC of the sophorolipid against *P. acne* was <1.5 mg/l and was lower than Irgasan DP-300. This result indicates that the sophorolipid could be used in health care products as an antimicrobial agent. Since sophorolipid has both surface-active property and antimicrobial activity, it has a potential as a cleansing product with antimicrobial activity, such as a body shampoo.

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