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Characteristics of Sophorolipid as an Antimicrobial Agent

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Abstract Sophorolipid, a biosurfactant produced from *Candida bombicola* ATCC 22214, showed antimicrobial activity against *Bacillus subtilis*, *Staphylococcus xylosus*, *Streptococcus mutans*, and *Propionibacterium acne* at 4, 1, 1, 0.5 ppm, respectively. Also, 100 ppm of sophorolipid inhibited 50% of cell growth of plant pathogenic fungus, *Botrytis cineria*. However, sophorolipid showed no effect on *Escherichia coli*, indicating that its selective antimicrobial activity depended on the cell wall structure. Treatment of *B. subtilis* with sophorolipid increased leakage of intracellular enzyme, malate dehydrogenase, indicating a possible interaction of sophorolipid with a cellular membrane. Comparing lactone-type and acid-type sophorolipids, the former showed a higher antimicrobial activity. Supplementing other surfactants showed no significant effects on the antimicrobial activity. Animal study showed that 5 g of sophorolipid per kg body weight by oral administration caused no toxicity, and sophorolipid induced no irritation on the skin. These results show potential use of sophorolipid as an active ingredient in healthcare products.

Key words: Antimicrobial activity, *P. acne*, biosurfactant, sophorolipid, MIC

Sophorolipid is a biosurfactant that is produced from *C. bombicola* [11, 36]. Biosurfactant consists of hydrophilic and hydrophobic groups in the same molecule that shows various types such as glycolipid, lipopeptide, and biopolymer [12, 21, 25, 26, 27, 46]. They are capable of producing unique chemical and biological properties, i.e., they are less toxic, more biodegradable, and less irritating to skin. Therefore, biosurfactants are gaining importance in areas such as food, biomedical, textile, cosmetics, and environmental cleanup [16, 17, 19, 36, 39, 47, 51] sciences.

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One of the unique properties of biosurfactant is its biological activity. Rhamnolipid, a glycolipid type biosurfactant that was produced from *Pseudomonas* sp., showed antimicrobial, antifungal, mycoplasmicidal, and antiviral activities [24, 45]. Surfactin and iturin, lipopeptide-type biosurfactants, inhibited bacterial cell growth by disintegrating the cellular membrane [1, 3] and showed some antiviral activity [24, 40]. Commercialization of biosurfactant as an antimicrobial agent is still limited due to the conventional chemical synthesis of antibiotics with low production costs. However, appearance of antibiotic-resistant pathogens increased the attention on other types of antimicrobial agents, such as natural products [13, 35].

Natural products with antimicrobial activities include peptides such as conalbumin, casein [4, 36], and organic acids which inhibit cell growth by decreasing the pH of culture broth or by blocking the substrate-uptake through the membrane [6].

Fatty acid showed effective growth-inhibition toward Gram-positive bacteria [10, 34]. Also, phytoalexin inhibited cell growth by alternating the cell membrane and inhibiting the electron transport in the mitochondria. [53]. Chitinase or β-1,3-glucanase effectively disintegrated fungal cell walls containing chitin and glucan [5]. Natural antimicrobial agents, as described previously, are mainly developed for inhibition of bacteria or fungi during cultivation or storage of agricultural products, and few studies have been reported on human health care.

Sophorolipid, a biosurfactant produced by *Candida* sp., is gaining attention, since it showed antimicrobial activity toward *P. acne*, an acne-inducing bacterium. Sophorolipid contained less irritating properties when compared to conventional antibiotics [34].

The sophorolipids were first discovered by Spencer [50] in 1954 and extensively investigated by Lang *et al.* [2, 7, 8, 27, 32, 33, 43, 44]. They elucidated the structure and showed that sophorolipid was produced as a mixture of

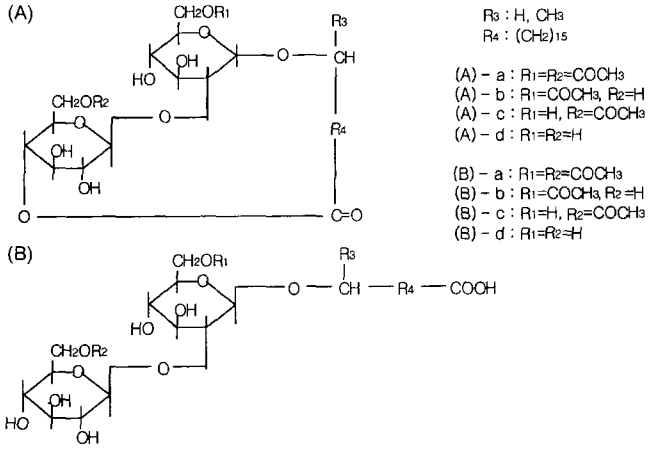

Fig. 1. Structure of sophorolipid; (a) lactone and (b) acid types (from Ref. 49).

lactone-type and acid-type as shown in Fig. 1 [49]. Among the biosurfactants reported in the literature, sophorolipid is the only biosurfactant that can be produced more than 300 g/l [44]. In addition, it can be produced from renewable resources such as whey [14].

Such economic advantages of sophorolipid along with its unique properties, which include high water-retention capability, water solubility, low toxicity to skin, encourage the application of sophorolipid as an antimicrobial agent in human health care products [11]. However, only a handful of studies reported on the properties of sophorolipid as an antimicrobial agent.

In this study, we investigated the effectiveness and characteristics of sophorolipid as an antimicrobial agent against various microorganisms.

MATERIALS AND METHODS

Production of Sophorolipid

Candida bombicola ATCC 22214 was stored on YM slants at 4°C. Production medium contained, per liter, 100 g of glucose, 5 g of yeast extract, 1 g of KH_2PO_4, 0.5 g of $MgSO_4$, 0.1 g of $CaCl_2 \cdot 2H_2O$, 0.1 g of NaCl, and 0.7 g of peptone. Cells grown for 48 h in 100 ml medium (500 ml flask) was inoculated into 2.5-liter fermentor jars (Kobiotech, Inchon, Korea) and cultivated for 7 days with 1-liter working volume, at 30°C, 550 rpm with 1 vvm of aeration without pH control. After cultivation, the bottom sophorolipid layer of culture broth was recovered by the gravitational separation process. The upper layer was centrifuged at 5,000 rpm for 30 min to remove cells and to obtain supernatant fraction. This fraction together with the bottom layer were extracted 3 times with an equal volume of ethyl acetate. After evaporating the ethyl acetate, hexane was added to remove the remaining oil. Finally, crude sophorolipid was extracted with chloroform to obtain a brown colored sophorolipid.

Fractionation of Sophorolipid Mixture

The sophorolipid sample was dissolved and eluted with solvent (chloroform:methanol:water=40:35:2, 2 ml/min) in a silica gel column (Merck, 230–400 mesh, 25 mm×50 mm). Two fractions of sophorolipid were separated in the column and recovered by evaporating the solvent, followed by an analysis by TLC/FID IATROSCAN (IATRON Co., Japan) and FT-IR (JASCO CO., Japan) for the structure identification. To carry out the TLC/FID analysis, 1 µl of the sample in methanol was loaded and eluted with same solvent that was used in the silica gel column. For the FT-IR analysis, 0.01 g of dehydrated sample was mixed with 1 g of KBr powder to form pellet and it was analyzed at 650–4,000 cm^{-1}.

Antimicrobial Activity Test

For the antibacterial test, all bacterial strains were obtained from KCTC. For *P. acne* cultivation, LAB M reinforced *clostridial* medium was used [20]. LB (Lenox L) 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 cultured at 37°C in a CO_2 incubator for 6–7 days, whereas other bacteria were incubated for 1–2 days. For antifungal activity, hyphae of *B. cineria* KCTC 6973 grown on the agar plate were cut into 5 mm discs, placed in the middle of the new agar plate and incubated for 3 days at 20°C. The diameter of colony was measured and the degree of inhibition was determined as the relative area of the colony [29].

Malate Dehydrogenase Assay

E. coli KCTC 1039 and *B. subtilis* KCTC 1028 were grown in LB medium, respectively. In the middle of the log phase, cells were harvested (5,000 rpm, for 10 min) and washed with saline. After being resuspended in phosphate buffer (pH 7, 100 mM), sophorolipid was added (300 ppm), and incubated for 2 h at 37°C. After centrifugation, the supernatant (50 µl) was added to 1 ml solution (0.2 mM of NADH, 0.33 of mM oxaloacetate, 94 mM of potassium phosphate) and the absorbance was measured at 340 nm. One unit of enzyme was defined as the amount of enzyme required for converting 1 µM of NADH into NAD [41].

Toxicity and Skin Irritation Tests

Toxicity and irritation tests were performed at the Veterinary School of Choongbuk Univeristy, Korea. Rats (Sprague-Dawley) were used for the toxicity test by oral administration according to the toxicity test protocol [18]. For skin irritation studies, rabbit (New Zealand White Rabbit) was used as a test animal.

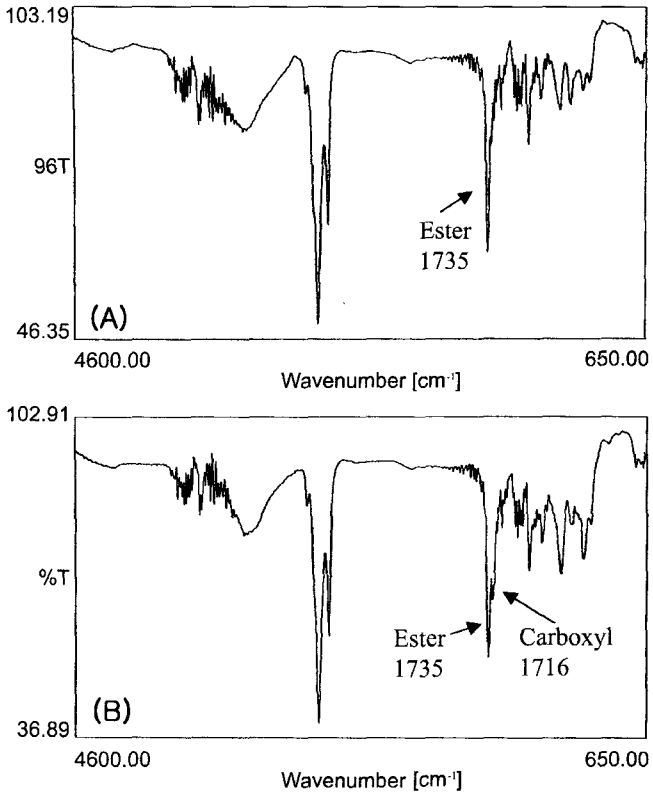

Fig. 2. FT-IR spectrum of sophorolipid.
(A) lactone type, (B) acid type.

RESULTS AND DISCUSSION

Identification of Sophorolipid Type

The FT-IR spectrum of carbonyl groups in the sophorolipid showed different absorption bands, i. e., 1,750–1,730 cm^{-1} for lactonic type and 1,725–1,700 cm^{-1} for acidic type [23]. By using this difference, the sophorolipid fraction that was isolated from the column was determined. As shown in Fig. 2, fraction A, showing its peak at 1,745 cm^{-1}, was identified as a lactonic type and fraction B, with 1,720 cm^{-1} peak, was determined as an acidic type. TLC/FID analysis showed that the ratio of lactonic and acidic types depended on the source of oil substrate used (Table 1). The ratio of two types was also reported to rely on the time of glucose addition during the cultivation for sophorolipid production [15].

Table 1. The effect of oil substrate on the type of sophorolipid.

	Lactone-type (%)	Acid-type (%)
Corn oil	14.5	85.5
Canola oil	5.5	94.5
Soybean dark oil*	40.3	59.7
Soybean oil	3.9	96.1

*A byproduct of soybean oil production.

Table 2. Antibacterial activity of the sophorolipid. [1]

Microorganism	Inhibitory concentration (ppm)[2]
Bacillus subtilis KCTC 1028	4
Staphylococcus xylosus ATCC 35663	1
Streptococcus mutans ATCC 35668	1
Propionibacterium acne ATCC 6919	0.5
Escherichia coli KCTC 1039	NI[3]

[1]Sophorolipid, produced from Canola oil, contained 95% acid-type.
[2]Inhibitory Concentration(IC) was defined in the Materials and Methods.
[3]NI, Not Inhibited.

Antimicrobial Activity Measurement

The inhibitory concentrations of sophorolipid, as shown in Table 2, were comparable to conventional antibiotics [22]. However, no inhibition of the cell growth for *E. coli* was observed, indicating its selective antimicrobial activity toward Gram-positive bacteria.

In order to investigate the effect of sophorolipid on cellular membrane, a leakage of cytoplasmic enzyme, by using the sophorolipid treatment, to the outside of the cell was measured. Malate dehydrogenase is a TCA cycle enzyme, found in animals, higher plants, and microorganisms [37, 42]. As shown in Table 3, a significant amount of malate dehydrogenase of *B. subtilis* was released into the medium after undergoing the sophorolipid treatment. However, little change was observed with *E. coli*. This indicated that sophorolipid increased the permeability of the membrane of Gram-positive bacteria. It was suggested that the lipopeptide, a biosurfactant molecule, might penetrate the cellular membrane, resulting in the leakage of cytoplasmic material and leading to cell lysis [40, 42]. Presumably, the protein and lipopolysaccharide in the cell walls of Gram-negative bacteria inhibited the penetration of sophorolipid molecules into the cellular membrane. To elucidate the interaction of sophorolipid with cellular membrane needs further work. Changes in the membrane composition and membrane structure are under investigation.

Antifungal activity of sophorolipid was investigated against *B. cineria*, a typical fungus causing gray, softened spots in apples, strawberries, and tomatoes. As shown in Table 4, 100 ppm of sophorolipid inhibited 50% of the fungal growth. This result shows a possibility that sophorolipid can actually replace the conventional and chemical fungicide currently used in the cultivation or storage of agricultural products.

Table 3. Release of intracellular enzyme with sophorolipid treatment.

Strain	Malate dehydrogenase activity (Unit/ml)	
	Before treatment	After treatment
E. coli	83	99
B. subtilis	25	158

Table 4. Antifungal activity of sophorolipid against *Botrytis cineria*.

Concentration (ppm)	Diameter of colony (cm)	Inhibition rate (%)
0	4.90	0
10	4.75	6
50	3.95	35
100	3.50	49
300	3.20	57

Inhibition rate $= \frac{D_o - D_i}{D_o} \times 100$

D_i; Diameter of colony (with sophorolipid).
D_o; Diameter of colony (without sophorolipid).

Low cost of production, low toxicity, antifungal activity as well as the biodegradable property of sophorolipid can mitigate the environmental and health related problems caused by calcitrant chemical fungicide.

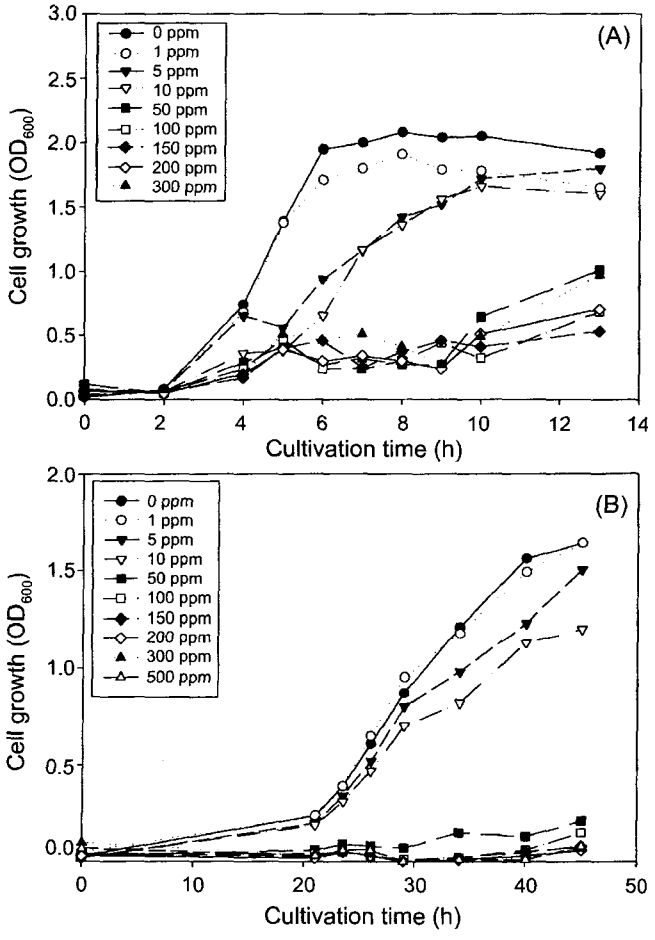

Fig. 3. Effect of sophorolipid on bacterial cell growth in the liquid medium.
(A) *Bacillus subtilis*, (B) *Propionibacterium acne*. Sophorolipid was added initially to the medium.

Growth Inhibition in the Liquid Culture

The effect of sophorolipid on bacteria growing in a liquid medium was investigated. As shown in Fig. 3A, 5 ppm of sophorolipid could decrease the growth rate of *B. subtilis* and no cell growth was observed above 150 ppm. Sophorolipid at 10 ppm decreased the growth rate of *P. acne* and a complete inhibition was observed at 50 ppm (Fig. 3B). In the liquid, surfactant molecules began to combine to form a micelle, a sphere-like structure with hydrophilic groups facing outside. This concentration is called a critical micelle concentration (CMC). The CMC of sophorolipid was 80 ppm. As shown in Fig. 3, growth inhibition was observed above 10 ppm. This result indicates that the mechanism of growth inhibition is not related to the micelle formation but with individual surfactant molecule.

Structure and Antimicrobial Activity

Two types of sophorolipid, lactone- and acid-type, were separated by silica gel chromatography and their antimicrobial activities were investigated. As shown in Fig. 4, lactone-

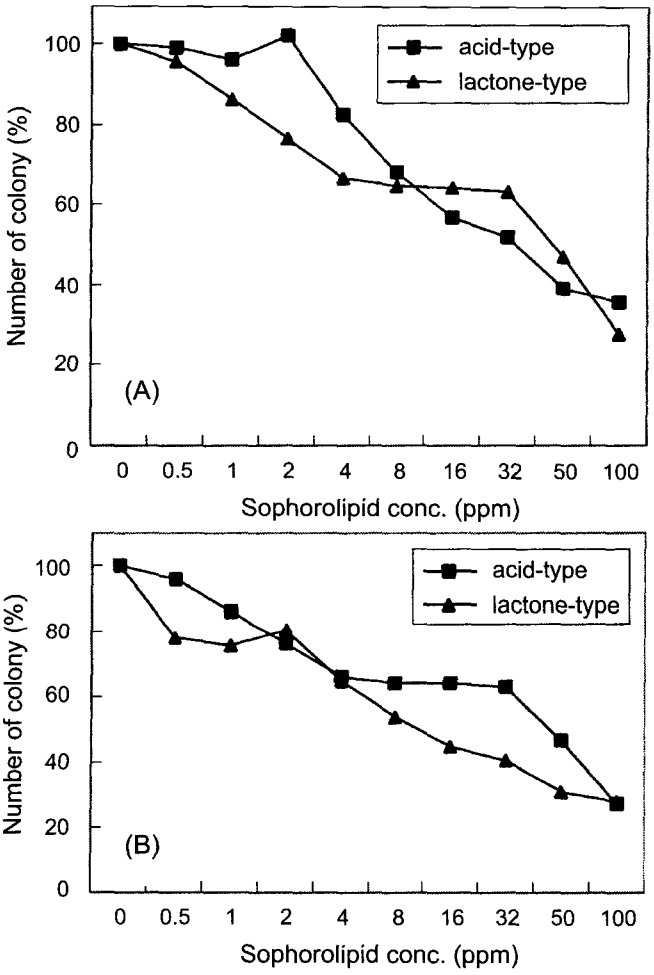

Fig. 4. Effect of sophorolipid type on bacterial cell growth.
(A) *Bacillus subtilis*, (B) *Propionibacterium acne*.

Table 5. Growth inhibition of *Propionibacterium acne* by sophorolippid (%).

	Sophorolipid	DSDM	PB	Sucrose monolaurate	Tween 80
50 ppm	100	96.1	21.7	8.63	1.6
300 ppm	100	100	91.8	98.9	0.5

DSDM; distearyl dimethyl ammonium chloride, PB; cocoamidopropyl betain, Tween 80; polyoxyethylene sorbitan monooleate.

type sophorolipid inhibited cell growth more effectively than acid-type for both *B. subtilis* and *P. acne*. Lang *et al.* [33] showed similar results. In the present work, we determined the inhibitory concentration (IC) of each sophorolipid type for both bacteria.

The ICs of lactone- and acid-type were 0.5 ppm and 16 ppm for *B. subtilis*, and 0.5 ppm and 4 ppm for *P. acne*, respectively. Lactone-type sophorolipid was more hydrophobic than the acid-type. Therefore, production of lactone-type sophorolipid by cultivation would be important and the factors influencing the ratio of lactone- and acid-type sophorolipids are under investigation in our laboratory. Since the sophorolipid is a surfactant, we compared its antimicrobial activity with other types of surfactants. As shown in Table 5, among the tested surfactants, sophorolipid inhibited *P. acne* most effectively. Although sucrose monolaurate has a similar structure as sophorolipid, sophorolipid actually showed a higher antimicrobial activity. It is interesting that inhibition of *P. acne* depends on the type of surfactant. Watanabe *et al.* [52] reported that the fatty acid moiety of surfactant affected the inhibition of cell growth. However, further works are required to elucidate the relationship between the structure of sophorolipid and its mechanism of cell growth inhibition.

The IC of Irgasan (3,5-Dichloro-N-(3,4-dichlorophenyl)-2-hydroxybenzamide) was compared with sophorolipid (Table 6). Irgasan is a commercial antimicrobial agent that is widely used in healthcare products such as toothpaste and soap. The IC of sophorolipid (0.5 ppm) was lower than that of Irgasan (3 ppm).

Table 6. Inhibition of *Propionibacterium acne* by sophorolipd and Irgasan (%).

Surfactant concentration (ppm)	Inhibition rate (%)	
	Sophorolipid	Irgarsan
0	0	0
0.5	13	0
1	-	0
2	26	0
3	-	7
4	-	11
5	-	29
6	45	-
8	48	-
10	44	84
50	100	100

Irgasan; (3,5-Dichloro-N-(3,4-dichlorophenyl)-2-hydroxybenzamide).

Effects of Various Surfactants on the Antimicrobial Activity of Sophorolipid

Various components including surfactants are mixed together during the formulation of cosmetic products such as a shampoo and a cream. Since sophorolipid is a surfactant, addition of other types of surfactants might influence the antimicrobial properties of sophorolipid. As shown in Table 7, no significant decrease or increase in antimicrobial activity was observed by adding various surfactants. As described previously (Fig. 3), it is a sophorolipid molecule, not a micelle, that alters the membrane structure. Therefore, inclusion of other surfactants might show less significant effects. If the micelle structure was required for the antimicrobial activity, addition of other surfactants would

Table 7. Effects of surfactant type on the antimicrobial activity of sophorolipid.

Type of surfactants	Surfactants	Inhibition rate (%)			
		Propionibacterium acne		*Bacillus subtilis*	
		Surfactant (20 ppm)	Surfactant (20 ppm) +Sophorolipid (20 ppm)	Surfactant (20 ppm)	Surfactant (20 ppm) +Sophorolipid (20 ppm)
	No surfactant	0.0	-	0.0	-
	Sophorolipid	90.0	-	37.7	-
Nonionic	Tween 81	0.0	90.7	5.0	37.0
	OA-20	0.0	43.3	13.0	17.0
	Span 80	53.3	95.0	21.0	60.0
	NP-8	73.3	99.0	-	-
Anionic	AOS-1416	-	-	2.0	81.0
Cationic	MPS-814S (EQ)	93.7	99.7	32.0	50.0
Zwitterionic	Cocamidopropyl-Betain	0.0	99.2	18.0	95.1

Tween 81: Polyoxyethylene sorbitan monooleate; OA-20: Polyoxiethylene oleylether; Span 80: Sorbitan monooleate; NP-8: Polyoxiethylene nonylpenylether; AOS-1416: α-oleffinsulfornate; MPS-814S: Esterquart.

probably influence the formation of sophorolipid micelle significantly and affect the antimicrobial activity.

Toxicity and Skin Irritation Tests

Toxicity or irritation of skin is an important property when looking at cosmetic ingredients. If the sophorolipid is to be used in a body cleanser, such as a shampoo, toxicity and skin compatibility should be evaluated. We have tested sophorolipid toxicity on 40 rats and 6 rabbits as test animals. When the sophorolipid was administered orally to the rat, no death was observed upto 5 g of sophorolipid per kg body weight. In fact, this indicated that sophorolipid was quite a safe ingredient, considering that the 100 ppm of sophorolipid completely inhibited the growth of *P. acne*.

Also, no irritation on skin was observed after 72 h. It is well known that some chemical surfactants showed a degree of skin irritation causing cracks in the hands [48]. Surfactants containing sugar moiety such as sugar ester were reported to be milder for skin irritation [38].

CONCLUSIONS

In this study, we showed that sophorolipid possessed antimicrobial activity against Gram-positive bacteria at low inhibitory concentration. The mechanism of antimicrobial action would be the alteration of membrane structure, since intracellular enzyme was released extracellularly upon sophorolipid treatment. When compared with other surfactants, sophorolipid showed a high capability for cell growth inhibition. Although the stability and effectiveness of sophorolipid should be investigated during the formulation procedure for commercialization, the low inhibitory concentration, low toxicity, and low cost of sophorolipid would be excellent properties for healthcare products, such as acne-curing soap, shampoo, and many other products.

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REFERENCES

1. Arima, K., A. Kakinuma, and G. Tamura. 1968. Surfactin, a crystaline peptide-lipid surfactant produced by *Bacillus subtilis*: Isolation, characterization and its inhibition of fibrin clot formation. *Biochem. Biophys. Res. Commun.* **31:** 488–494.
2. Asmer, H.-J., S. Lang, F. Wagner, and V. Wray. 1988. Microbial production, structure and elucidation and bioconversion of sophorose lipids. *J. Am. Oil Chem. Soc.* **65:** 1460–1466.
3. Berbheimer, A. W. and L. S. Avigad. 1970. Nature and properties of a cytological agent produced by *Bacillus subtilis*. *J. Gen. Microbiol.* **61:** 361–369.
4. Bezkorovainy, A. 1981. Antimicrobial properties of iron-binding proteins. *Adv. Exp. Med. Biol.* **135:** 139–154.
5. Bhushan, B. and G. S. Hoondal. 1999. Effect of fungicides, insecticides and allosamidin on a thermostable chitinase from *Bacillus* sp. BG-11. *World J. Microbiol. Biotechnol.* **15:** 403–404.
6. Blanc, P. and G. Goma. 1987. Kinetics of inhibition in propionic acid fermentation. *Bioprocess Eng.* **2:** 175–179.
7. Brakemeier, A., D. Wullbrandt, and S. Lang. 1998. *Candida bombicola*: Production of novel alkyl-glycosides based on glucose/2-dodecanol. *Appl. Microbiol. Biotechnol.* **50:** 161–166.
8. Brakemeier, A., D. Wullbrandt, and S. Lang. 1998. Microbial alkyl-sophorosides based on 1-dodecanol or 2-, 3-, and 4-dodecanones. *Biotechnol. Lett.* **20:** 215–218.
9. Brakemeier, A., S. Lang, D. Wullbrandt, L. Merschel, A. Benninghoven, N. Buschmann, and F. Wagner. 1995. Novel sophorose lipids from microbial conversion of 2-alkanols. *Biotechnol. Lett.* **17:** 1183–1188.
10. Ching. J. U., W. Sheu, and E. Freese. 1972. Effects of fatty acids on growth and envelope proteins of *Bacillus subtilis*. *J. Bacteriol.* **111:** 516–524.
11. Cho, K.-J., Y. B. Kim, and E. K. Kim. 1999. Production and application of sophorolipid, a microbial surfactant. *Kor. J. Biotechnol. Bioeng.* **14:** 747–753.
12. Choi, K.-S., S. H. Kim, and T. H. Lee. 1999. Purification and characterization of biosurfactant from *Tsukamurella* sp. 26A. *J. Microbiol. Biotechnol.* **9:** 32–38.
13. Cooper, A. J. 1998. Systematic review of *Propionibacterium acne* resistance to systemic antibiotics. *Med. J. Australia* **169:** 259–261.
14. Daniel, H. J. and R. T. Otto. 1999. Production of sophorolipids from whey: Development of a two-stage process with *Cryptococcus curvatus* ATCC 20509 and *Candida bombicola* ATCC 22214 using deproteinized whey concentrates as substrates. *Appl. Microbiol. Technol.* **25:** 613–621.
15. Davila, A. M., R. Marchal, and J. P. Vandecasteele. 1997. Sophorose lipid fermentation with differentiated substrate supply for growth and production phases. *Appl. Microbiol. Biotechnol.* **47:** 496–501.
16. Desai, J. P. and I. M. Banat. 1997. Microbial production of surfactants and their commercial potential. *Microbiol. Mol. Rev.* **61:** 47–64.
17. Finnerty, W. R. 1994. Biosurfactant in environmental biotechnology. *Curr. Opin. Biotechnol.* **5:** 291–295.
18. Food and Drug Association, 1999. *Standard Methods for Toxicity Test of Pharmaceuticals*; 1999-61, Korea.
19. Gang, J.-G., H. C. Yun, J. C. Son, and S. Y. Hwong. 1998. Effect of 1-[(2-hydroxyethoxy) methyl]-6-(phenylthio) thymine (HEPT) derivatives on bacterial growth. *J. Microbiol. Biotechnol.* **8:** 229–236.

20. Gribbon, E. M., W. J. Cunliffe, and K. T. Holland. 1993. Interaction of *Propionibacterium acnes* with skin lipids *in vitro*. *J. Gen. Microbiol.* **139:** 1745–1751.
21. Han, C.-S., H. S. Yun, H. J. Seo, and E. K. Kim. 1999. Biosurfactant production from phenanthrene degrading bacteria. *Kor. J. Biotechnol. Bioeng.* **14:** 737–741.
22. Hommel, R. K., O. Stuwer, W. Stuber, D. Haferberg, and H. P. Kleber. 1987. Production of water-soluble surface active exolipids by *Torulopsis apicola*. *Appl. Microbiol. Biotechnol.* **26:** 199–205.
23. Ito, S. and S. Inoue. 1982. Sophorolipids from *Torulopsis bombicola*: Possible relation to alkane uptake. *Appl. Environ. Microbiol.* **43:** 1278–1283.
24. Itokawa, H., T. Miyashita, H. Morita, K. Takeya, T. Hirano, M. Homma, and O. Kitaro. 1994. Structural and conformational studies of Ile^7 and Leu^7 surfactant from *Bacillus subtilis*. *Chem. Pharm. Bull.* **42:** 604–607.
25. Jung, H.-K., J. B. Lee, G. B. Yim, and E. K. Kim. 1995. Properties of microbial surfactant, S-acid. *Kor. J. Biotechnol. Bioeng.* **10:** 71–77.
26. Kim, D.-H., J. B. Lee, G. B. Yim, and E. K. Kim. 1995. Optimized production of microbial surfactant, S-acid, from *Penicillium spiculisporum*. *Kor. J. Biotechnol. Bioeng.* **10:** 113–118.
27. Kim, J.-S., M. Powalla, S. Lang, F. Wagner, H. Lünsdorf, and V. Wray. 1990. Microbial glycolipid production under nitrogen limitation and resting cell conditions. *J. Biotechnol.* **13:** 257–266.
28. Kim, S.-H., J. D. Lee, B. C. Lee, B. C. Kim, and T. H. Lee. 1996. Purification and characterization of bioemulsifier produced by *Acinetobacter* sp. BE-254. *J. Microbiol. Biotechnol.* **6:** 184–188.
29. Koh, K. H. 1995. Antifungal activity of caproic acid against *Botrytis cinerea*. *Kor. J. Appl. Microbiol. Biotechnol.* **23:** 378–383.
30. Kosaric, N., W. L. Cairns, N. C. C. Gray, D. Stechey, and J. Wood. 1984. The role of nitrogen in multiorganism strategies for biosurfactant production. *J. Am. Oil Chem. Soc.* **61:** 1735–1743.
31. Lahov, E. and W. Regelson. 1996. Antibacterial and immunostimulating casein-derived substances from milk: Casecidin, isracidin peptides. *Food Chem. Toxicol.* **34:** 131–145.
32. Lang, S., A. Brakemeier, A. Schlotterbeck, and F. Wagner. 1996. Biotechnological synthesis of surface-active glycolipids. *Euro Cosmetics* **4:** 41–45.
33. Lang, S., E. Katsiwela, and F. Wagner. 1989. Antimicrobial effects of biosurfactants. *Fat Sci. Technol.* **91:** 363–366.
34. Lee, B.-Y., I. H. Yoon, Y. B. Kim, P. J. Han, and C. M. Lee. 1985. Studies on storing chest-nut (*Castanea crenata* var. dulcis Nakai) sealing with polyethylene film. *Korean J. Food Sci. Technol.* **17:** 331–335.
35. Lee, H.-Y., C. K. Kim, T. K. Sung, T. K. Mun, and C. J. Lim. 1992. Antibacterial activity of *Ulmus pumila* L. extract. *Kor. J. Appl. Microbiol. Biotechnol.* **20:** 1–5.
36. Lin, S. C. 1996. Biosurfactants: Recent advances. *J. Chem. Tech. Biotechnol.* **66:** 109–120.
37. Miller, S. S., B. T. Driscoll, R. G. Gregerson, J. S. Gantt, and C. P. Vance. 1998. Alfalfa malate dehydrogenase MDH molecular cloning and characterization of five different forms reveals a unique nodule-enhanced MDH. *Plant J.* **15:** 173–184.
38. Mitsubishi-Kasei Food Corporation, 1987. Ryoto Sugar Ester, Technical Information, Mitsubishi-Kasei Food Corporation, Japan.
39. Muller-Hurtig, R., F. Wagner, R. Blaszczyk, and N. Kosaric. 1993. Biosurfactants for environmental control, pp. 447–469. *In* N. Kosaric (ed.), *Biosurfactant - Production, Properties and Applications*. Surfactant Science Series, Vol. 48. Marcel Dekker, New York, U.S.A.
40. Naruse, N., O. Tenmyo, S. Kobaru, H. Kamai, T. Miyaki, M. Konishi, and T. Oki. 1990. Pumilacidin, a complex of new antiviral antibiotics: Production, isolation, chemical properties, structure and biological activity. *J. Antibiot.* **43:** 267–280.
41. Nikolaos, E. L. and D. C. Yannis. 1997. L-Malate dehydrogenase from *Pseudomonas stuzeri*: Purification and characterization. *Archives Biochem. Biophys.* **337:** 103–114.
42. Ocheretina, O. and R. Scheibe. 1997. Cloning and sequence analysis of cDNAs encoding plant cytosolic malate dehydrogenase. *Gene* **199:** 145–148.
43. Rau, U., R. Heckmann, V. Wray, and S. Lang. 1999. Enzymatic conversion of a sophorose lipid into a glucose lipid. *Biotechnol. Lett.* **21:** 973–977.
44. Rau, U., S. Hammen, R. Heckmann, V. Wray, and S. Lang. 2001. Sophorolipids: A source for novel compounds. *Ind. Crops Products* **13:** 85–92.
45. Stanghellini, M. E. and R. E. Miller. 1999. Biosurfactants: Their identity and potential efficacy in the biological control of zoosporic plant pathogens. *Plant Dis.* **81:** 4–12.
46. Suk, W.-S., H. J. Son, G. Lee, and S. J. Lee. 1999. Purification and characterization of biosurfactants produced by *Pseudomonas* sp. SW1. *J. Microbiol. Biotechnol.* **9:** 56–61.
47. Sullivan, E. R. 1998. Molecular genetics of biosurfactant production. *Curr. Opin. Biotechnol.* **9:** 263–269.
48. Tagesson, C. and C. Edling. 1984. Influence of surface-active food additives on the integrity and permeability of rat intestinal mucosa. *Food Chem. Toxicol.* **22:** 861–864.
49. Tulloch, A. P., A. Hill, and J. F. T. Spencer. 1968. Structure and reactions of lactonic and acidic sophorosides of 17-hydroxy octadecanoic acid. *Can. J. Chem.* **46:** 3337–3351.
50. Tulloch, A. P. 1976. Structures of extracellular glycolipids produced by yeasts, pp. 329–345. *In* L. A. Witting (ed.), *Glycolipid Methodology*. American Oil Chemists Society, Champaign, IL, U.S.A.
51. Velikonja, J. and N. Kosaric. 1993. Biosurfactants in food application, pp. 419–446. *In* N. Kosaric (ed.), *Biosurfactant - Production, Properties and Applications*. Marcel Dekker, New York, U.S.A.
52. Watanabe T., S. Katayama, M. Matsubara, Y. Honda, and M. Kuwahara. 2000. Antibacterial carbohydrate monoesters suppressing cell growth of *Streptococcus mutans* in the presence of sucrose. *Curr. Microbiol.* **41:** 210–213.
53. Zheng, J. and V. D. Ramirez. 2000. Inhibition of mitochondrial proton F0F1-ATPase/ATP synthase by polyphenolic phytochemicals. *Br. J. Pharmacol.* **130:** 1115–1123.