J. Microbiol. Biotechnol. (2010), **20**(12), 1664–1671
doi: 10.4014/jmb.1007.07052
First published online 9 October 2010

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Bioproduction and Anticancer Activity of Biosurfactant Produced by the Dematiaceous Fungus *Exophiala dermatitidis* SK80

Chiewpattanakul, Paramaporn[1,2], Sirinet Phonnok[1], Alain Durand[2], Emmanuelle Marie[2], and Benjamas Wongsatayanon Thanomsub[1]*

[1]*Department of Microbiology, Faculty of Medicine, Srinakharinwirot University, Bangkok 10110, Thailand*
[2]*Laboratoire de Chimie Physique Macromoléculaire CNRS-Nancy University, ENSIC, 1 rue Grandville, BP 20451, F-54001 Nancy cedex, France*

Received: July 23, 2010 / Revised: August 29, 2010 / Accepted: August 30, 2010

A new biosurfactant producer was isolated from palm-oil-contaminated soil and later identified through morphology and DNA sequencing as the yeast-like fungus *Exophiala dermatitidis*. Biosurfactant production was catalyzed by vegetable oil, supplemented with a basal medium. The culture conditions that provided the biosurfactant with the highest surface activity were found to be 5% palm oil with 0.08% NH_4NO_3, at a pH of 5.3, with shaking at 200 rpm, and a temperature of 30°C for a 14-day period of incubation. The biosurfactant was purified, in accordance with surfactant properties, by solvent fractionation using silica gel column chromatography. The chemical structure of the strongest surface-active compound was elucidated through the use of NMR and mass spectroscopy, and noted to be monoolein, which then went on to demonstrate antiproliferative activity against cervical cancer (HeLa) and leukemia (U937) cell lines in a dose-dependent manner. Interestingly, no cytotoxicity was observed with normal cells even when high concentrations were used. Cell and DNA morphological changes, in both cancer cell lines, were observed to be cell shrinkage, membrane blebbling, and DNA fragmentation.

Keywords: Monoolein, monoglyceride, biosurfactant, anticancer, microbial product, *Exophiala*

Biosurfactants are surface-active agents produced by the biological processes of microorganisms as membrane components or excretions [17]. The properties of biosurfactants, as amphipathic molecules, make them good mediators in emulsification processes in various applications. Oil-contaminated samples are favorable sites for the screening of biosurfactant producers. Oil enhances the biosurfactant synthesis of these microorganisms, which assimilate the oil into small droplets and then digest these oil droplets for their energy [5]. As biosurfactants are produced through biological processes, their medical use is advantageous owing to their structural diversity, biodegradability, reduced toxicity [6], low irritancy, and compatibility with human skin [16]. They have also been used for many other purposes such as food additives (emulsifiers) in the food industry, herbicides and pesticides in the agricultural industry, and even for bioremediation, cosmetics, and pharmaceuticals [17]. In their pharmaceutical applications, there have been reports claiming that biosurfactants exhibit biological activities in the form of antibiotic, antiviral, and antifungal effects [2].

Cancer is a notorious disease that poses many complications in treatment owing to issues of drug efficacy and harmful side effects for normal cells. A major challenge in the discovery of a drug with the potential to cure cancer is to find an efficient drug with a minimal toxicity to normal cells. This research describes the isolation of a microorganism that produces a biosurfactant from oil-contaminated soil, collected from palm oil factory waste, and the optimization of culture conditions in order to improve production yield. The biosurfactant was then purified, its structure investigated and elucidated, and its potential anticancer activity explored.

Materials and Methods

Isolation and Identification of Biosurfactant Producer

The microorganisms were isolated from oil-contaminated soils collected from oil mill wastes using basal medium agar plates

**Corresponding author*
Phone: +66-2-649-5000; Fax: +66-2-649-5394;
E-mail: benjamat@swu.ac.th

containing NH_4NO_3 (3 g/l), KH_2PO_4 (0.2 g/l), $MgSO_4$ (0.2 g/l), yeast extract (0.5 g/l), and 0.1% (v/v) Tween 20 supplemented with 1% (v/v) palm oil. The isolated microorganisms were tested for surfactant activity and emulsion activity. The strain that yielded the highest biosurfactant and emulsion activities was selected, grown on YM agar plates, and identified by macro- and microscopic morphology. The D1/D2 region of the 28S rRNA was amplified, sequenced, and analyzed by Gapped BLAST and PSI-BLAST [1]. The genetic analysis was performed at the Mahidol University–Osaka University Collaborative Research Center for Bioscience and Biotechnology (MU–OU: CRC), Thailand.

Surfactant Activity and Emulsion Activity Assays

The culture broth supernatants were screened for biosurfactant production capability by drop-collapse test [4]. The emulsification activity was determined by the modified method of Patel and Desai [15]. Assays were carried out in triplicate. In brief, 1 ml of kerosene was added to 1 ml of cell-free supernatant broth and mixed (Vortex genie2, USA.) at a high speed for 2 min. The height of the interface emulsion layer and the total height were measured at 24 h and 48 h, and calculations for emulsifying activity and stability were performed using the following formula:

$$\text{Emulsion activity/stability (\%)} = \frac{\text{height of emulsion layer}}{\text{total height}} \times 100$$

Optimization of Culture Conditions

The basal medium (BM) used in this study consisted of 0.3% NH_4NO_3, 0.02% KH_2PO_4, 0.02% $MgSO_4.7H_2O$, and 0.05% yeast extract, at a pH of 5.3. To study the effect of carbon sources, glucose or various types of oil was supplemented into the basal medium at 4%. The types of carbon sources giving the highest surfactant activity were selected and studied for optimum concentrations, in a band ranging from 3–8%. The minimum concentration of carbon source that gave the highest biosurfactant production was then chosen for further study on the optimization of nitrogen sources. First, 0.4 ml of the spore stock was added to 20 ml of the tested medium prepared in a 100-ml flask, and then shaken at 200 rpm at 30°C (Thermo Fisher Scientific Inc., USA.). The culture broth was taken daily in order to determine the surfactant activity. Cell growth was measured by the optical density of the culture broth at 600 nm with a UV–Visible spectrophotometer (Shimadzu UV-160A, Japan). Cultivation and the activity assays were performed in three independent experiments, and the data were calculated from triplicate determinations of surfactant activity. Statistically significant differences of tested media and culture conditions were evaluated by one way ANOVA ($P<0.05$) using SPSS 10 software.

Extraction and Purification of Biosurfactant

SK80 was cultivated in a 6 l-basal salt medium with optimum culture conditions at 30°C for 14 days. The resulting broth was extracted by ethyl acetate and evaporated. The residue was dissolved with methanol and washed twice with hexane so as to obtain the crude extract. The crude extract was then purified by silica gel chromatography using sequential elution with hexane, choloroform, ethyl acetate, and methanol. All fractions were collected, dried, and tested for their surfactant activities. The fraction that exhibited the highest surfactant activity was then repeatedly purified until the pure compound was obtained.

Analytical Methods

The crude extract and purified products were analyzed by thin-layer chromatography (TLC) and visualized by ρ-anisaldehyde spray. The chemical structure of the purified compound was determined by ^1H NMR, ^{13}C NMR, HSQC, and COSY (BRUKER spectrometer Avance 300) in deuterated chloroform ($CDCl_3$) and through an atmospheric pressure chemical ionization mass spectrometer (APCI MS).

Cell Lines and Culture Conditions

The cervical cancer (HeLa), liver cancer (HepG2), breast cancer (MCF-7), monocytic leukemia (U937), and African green monkey kidney (Vero) cell lines originated from the American Type Culture Collection (ATCC). The medium used for the HepG2, MCF-7, and HeLa cell lines was Dulbecco's modified Eagle's medium (DMEM; GIBCO, USA), for U937 cells was RPMI1640, and for Vero cells was M199. All cells were supplemented with 10% fetal bovine serum and 100 U/ml of penicillin–streptomycin and incubated at 37°C with 5% CO_2. Whole blood was taken from 3 healthy individual subjects and peripheral blood mononuclear cells (PBMC) were separated using a Ficoll Plague Premium reagent kit (GE Healthcare Bio-Sciences AB, Sweden) and maintained in an RPMI medium.

Cytotoxicity Assay

Aliguots (90 µl) containing 1.0×10^4 cells of cell line suspensions (MCF-7, HepG2, HeLa, U937, Vero cells) were seeded into the wells of 96-well plates. After 24 h of incubation, 10 µl of crude extracts dissolved in 0.01% ethanol was added to the final concentrations of 500 or 1,000 µg/ml, and incubated for a further 48 h. The cytotoxicity test was conducted through the use of an MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay [8], which detected the formazan dye at 595 nm using a microplate spectrophotometer (Multiskan, Finland). Each treatment of SK80 crude extract and pure biosurfactant was assayed in triplicate. As a vehicle control, 0.01% ethanol was used as the negative control. Statistical analysis between treatment and control was determined using the paired two-tailed Student's *t*-test. *P*-values of <0.05 were considered to be significantly different from the control group. The cytotoxicity of the crude extract was expressed as a value of IC_{50} (where concentrations exhibited 50% cytotoxicity). The significant differences in IC_{50} of crude extracts, against the same cell lines, were compared using the one-way ANOVA, PostHoc test.

Morphology of Cell Death Observations

Concentrations levels that caused 70% cell death were used; 70 µg/ml of SK80 crude extract with HeLa, and 40 µg/ml of pure biosurfactant or 60 µg/ml of monoolein with U937. For normal cells (Vero and PBMC), 1,000 µg/ml of monoolein was used. The cells, treated with or without microbial crude extracts or monoolein, were incubated at 37°C for 48 h, washed 3 times with PBS, and then observed under a phase-contrast inverted microscope (Nikon, Japan). For DNA staining experiments, 50 µl of 100 µg/ml Hoechst 33342 was added after washing, incubated at 37°C for 10 min, and examined under a fluorescence inverted microscope at an emission wavelength of 461 nm (Olympus, Japan).

DNA Ladder Assay

Cancer cells, at 3×10^6 cells, were treated with SK80 extract or monoolein, at concentrations found to have induced 70% cytotoxicity,

and incubated at 37°C with 5% CO_2 for 24 h. At the end of this incubation period, the chromosomal DNA of cancer cells was prepared with an Apoptotic DNA Ladder kit (Roche, Cat. No. 1835246001). Cells were harvested and lysed with a lysis buffer for 10 min. Then the samples were mixed with isopropanol before being passed through a filter and washed. The DNA was then eluted from the filter and treated with RNAse at 37°C for 30 min before loading onto a 2% agarose gel for electrophoresis, run at 50 V/cm for 3 h. The normal cells were also examined in parallel.

RESULTS AND DISCUSSION

Isolation and Identification of Biosurfactant Producer Strain

Amongst the 102 isolates, the biosurfactant-producing microorganism, strain SK80, isolated from soil collected from palm oil factory waste in the Songkhla province of Thailand, exhibited the highest surfactant and emulsification activity. At initial growth on YM agar, the SK80 strain appeared as a brownish-black, yeast-like colony, and after day 5, it became an olivaceous grey, suede-mold-like colony. Correspondingly, the microscopic study of SK80, at initial growth, showed a yeast-like cell, unicellular in structure, and ovoid to elliptical in shape, with a budding hyaline and thin wall. On days 5–7, it became darkly pigmented (dematiaceous) and thick-walled. At the mold stage, flask-shaped to cylindrical phialides, without distinctive collarettes, were produced. Conidia were observed to be hyaline to pale brown, with a round to ovoidal cell (data not shown).

The results of D1/D2 sequencing, and of 28S rRNA similarity searches, alongside the morphological study, suggested that SK80 is *Exophiala dermatitidis*. This is a dematiaceous yeast-like fungus, which is commonly found in the environment, in particular in plant debris and soil, in both temperate as well as tropical climates. It is also known to exist in the man-made environment, such as in human feces, oil debris, and steam baths [21]. However, this is the first report of the isolation of the biosurfactant produced from this strain.

Time Course and Biosurfactant Production

Biosurfactants are a group of structurally diverse molecules produced by very different kinds of microorganisms. Several factors affect biosurfactant production. These factors include carbon sources, nitrogen sources, and more general environmental growth conditions. The study on the growth and biosurfactant production of SK80 was monitored in basal salt media supplemented with a number of various carbon sources. Surfactant activity was detected in the vegetable-oil-supplemented groups of soybean oil, corn oil, and palm oil. Basal culture media without a carbon source (no oil), as well as those supplemented with glycerol, glucose, or *n*-hexadecane, were found not to be able to induce biosurfactant production (Fig. 1). Surfactant activity values obtained from all days of C-source cultivation were statistically tested, and palm oil was shown to induce the highest surfactant activity, giving the most significant difference at $P<0.05$. In addition, palm oil is the cheapest amongst the various vegetable oils used. The optimum concentration of palm oil was investigated and it was shown that a culture broth cultivated using a 5% palm oil concentration exhibited the highest surfactant activity (data not shown). This culture broth was used for subsequent investigations. Biosurfactant production from bacteria and yeast can be naturally synthesized, or induced by hydrocarbon, glucose, or vegetable oil. In addition, carbohydrates and vegetable oils are among the most widely used substances for research on biosurfactant production for *Pseudomonas aeruginosa* strains [17, 13, 23]. In this study, the biosurfactant production of *Exophiala dermatitidis* SK80 was also specifically induced through vegetable oil.

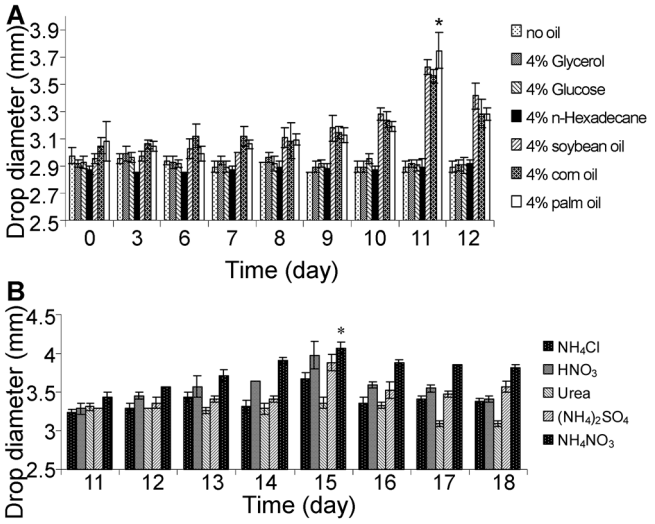

Fig. 1. Effects of type of (**A**) carbon source and (**B**) nitrogen source used for SK80 cultivation on surfactant activity.
The conditions used for cultivation in **A** were a basal medium supplemented with various types of carbon sources, with shaking at 200 rpm at 30°C. In **B**, the culture conditions were a basal medium plus 5% palm oil and varying types of nitrogen source. *Significant differences, *P*-value <0.05.

NH_4Cl, HNO_3, $(NH_4)_2SO_4$, NH_4NO_3, and urea were used in the experiments as nitrogen sources. Urea was found to be unable to induce biosurfactant production; on the other side of the scale, NH_4NO_3 was shown to induce the highest surfactant activity. It was notable that, in all days tested, there were significant differences between activity when using other nitrogen sources (Fig. 2B). The optimum concentration of NH_4NO_3 was 0.08% (data not shown). The nitrogen source is an important factor for the production of biosurfactants; nevertheless, a limitation in

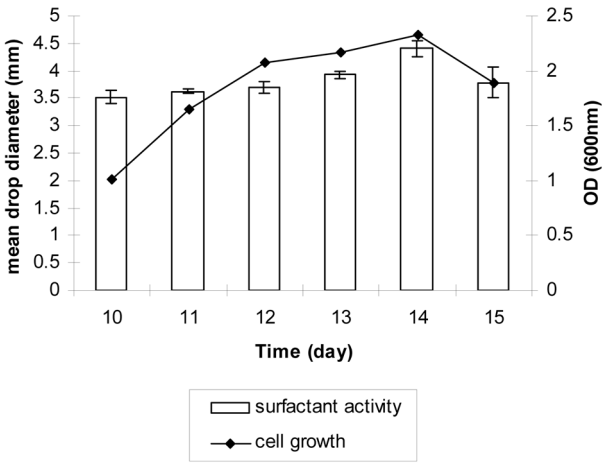

Fig. 2. Time course of cell growth and biosurfactant production of *Exophiala dermatitidis* SK80 under optimal culture conditions, which were a basal medium (0.02% KH_2PO_4, 0.02% $MgSO_4 \cdot 7H_2O$, 0.05% yeast extract) supplemented with 5% palm oil, 0.08% NH_4NO_3, pH 5.3, and shaking at 200 rpm at 30°C.

nitrogen sources can lead to an increase in biosurfactant production in some cases, such as with *Nocardia* sp., *Candida tropicalis* [14, 20], and in our study The production of biosurfactant, and growth of *Exophiala dermatitidis* SK80 under optimal conditions, correlated with one another (Fig. 2). Cell growth increased between days 10 and 14, and decreased on day 15, which matched the changes in levels of biosurfactant production. The highest values in both surfactant activity and cell growth were reached when the cultivation was 14 days old. The surfactant activity of the culture supernatant obtained from the optimized culture was 1.2 times higher, with emulsifying activity and emulsion stability being 2 times higher, than the culture supernatant obtained under normal culture conditions (Table 1).

Purification of Biosurfactant Produced by *Exophiala dermatitidis*

Exophiala dermatitidis was cultivated in a 6 l basal salt medium under optimal culture conditions (5% palm oil, 0.08% NH_4NO_3, pH 5.3, and 30°C). The culture broth was extracted with ethyl acetate and evaporated. The extract (239.86 g) obtained from the ethyl acetate fraction was then dissolved in methanol/hexane at a ratio of 1:2, and a yellowish-brown crude extract was obtained from the methanol fraction. Approximately 24 g of the collected crude extract was then purified by silica gel column chromatography. The pure compound was eluted with chloroform:ethyl acetate at a ratio of 4:1 (v/v), revealing a band at an Rf value of 0.34 on TLC [hexane:ethyl acetate, 1/1 (v/v) mixture] (Fig. 4). The yield of purified biosurfactant (0.2 g) was 0.08% or 0.83% of crude extract when obtained from ethyl acetate and methanol, respectively. The use of vegetable oil as a C-source required the additional step of oil washing, and thus involved a greater loss of the biosurfactant.

We examined the origin of the biosurfactant by comparing the crude extract obtained from the basal medium, supplemented with 5% palm oil, with and without SK80 inoculum, and the purified compound on TLC. There was observed to be no band at Rf 0.34, which was the position of that of the purified biosurfactant in the uninoculed medium extract (data not shown), suggesting that the bioproduction of biosurfactant was indeed from the cultivation of the SK80 strain. It is noteworthy that vegetable palm oil contains less than 0.3% monoglycerides [9], and that the processes of agitation and crude extraction also imply the loss of some fatty acids and monoglycerides.

Structure Elucidation of the Pure Biosurfactant

The chemical structure of the purified biosurfactant (SK80) was characterized by ^{1}H NMR (Fig. 3A), ^{13}C NMR (Fig. 3B), HSQC (Fig. 3C), and COSY (Fig. 3D). NMR spectra were obtained in $CDCl_3$. The ^{1}H NMR spectrum exhibited signals for the alkyl chain at 0.9 ppm for the $-CH_3$ group, 1.3–2.4 ppm for the $-CH_2$ group, and 5.4 ppm for the –CH= group. For the glycerol moiety, the results were 3.6 ppm for the $-CH_2$ group, 3.9 ppm for the –CH group, and 4.1 ppm for the $-CH_2$ group. The ^{13}C NMR spectrum of SK80 revealed signals at 14.8 ppm for $-CH_3$, at 25.6, 27.8, 30.0, and 35.0 ppm for $-CH_2$, and at 130.7 for –CH= in the alkyl chain. Signals were noted for $-CH_2$ at 64.0 and 65.8, for the –CH group at 71.0 of the glycerol moiety, and at 175.0 for C=O of the ester. HSQC and COSY experiments confirmed these attributes. Aside from the main product, NMR spectra revealed the presence of a minor product that could not be identified.

Table 1. Surfactant and emulsification index of the culture broth supernatant of SK80 before and after optimization of the culture condition.

	Before optimization[a]	After optimization[b]	Medium control (BM+4% soybean oil)
Surfactant activity (mm)	3.62±0.11	4.40±0.14	2.90±0.04
Emulsion activity (%, 24 h)	41.38±0	81.82±0	0
Emulsion stability (%, 48 h)	41.38±0	81.82±0	0

[a]BM supplemented with 4% soy bean oil, 0.03% NH_4NO_3, pH 5.3, 200 rpm at 30°C.
[b]BM supplemented with 5% palm oil, 0.08% NH_4NO_3, pH 5.3, 200 rpm at 30°C.

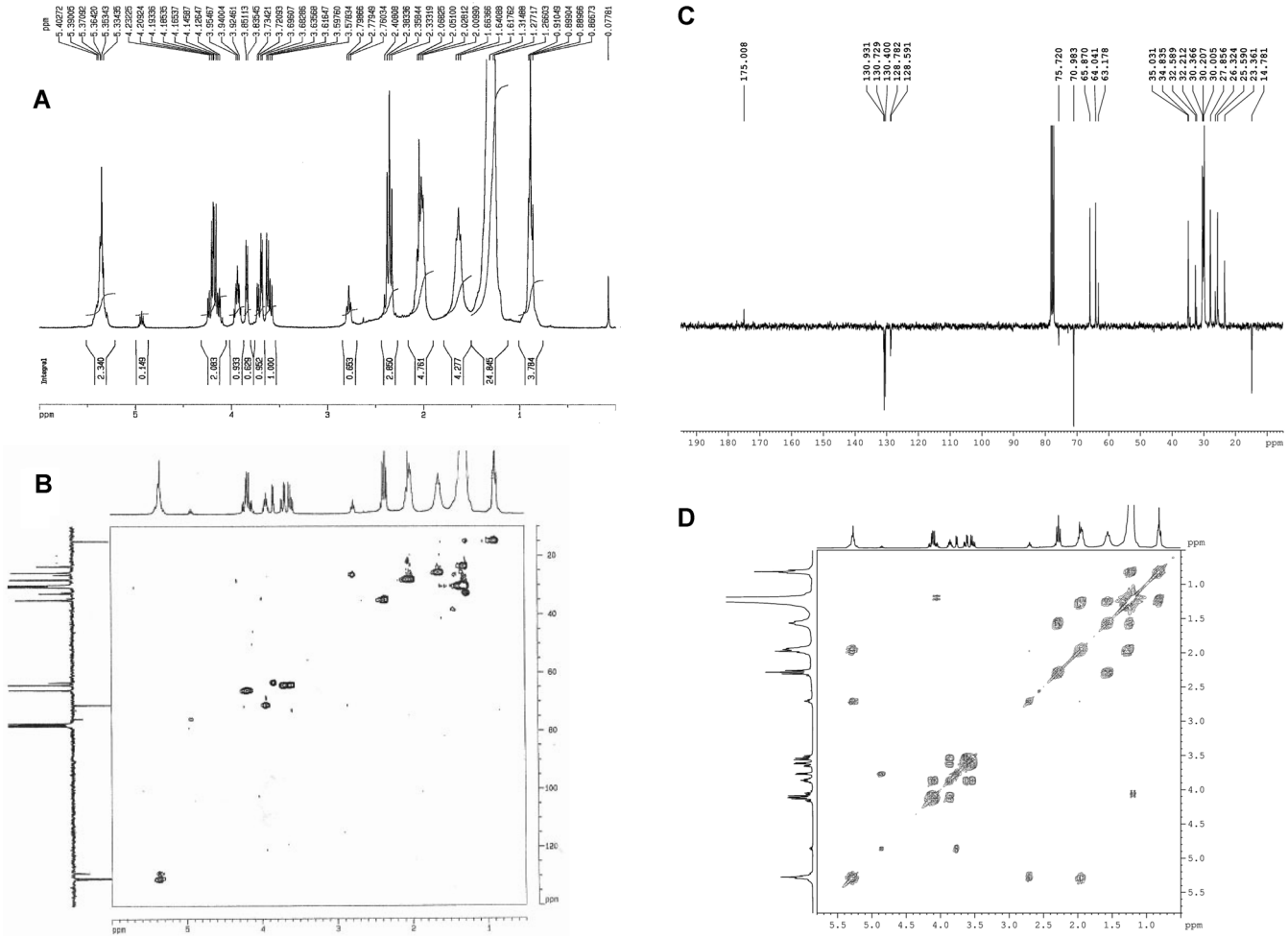

Fig. 3. Analytical spectra of the purified.
A. ^{1}H NMR spectrum of purified biosurfactant, in $CDCl_3$, 300 MHz; **B**. ^{13}C NMR spectrum of purified biosurfactant, in $CDCl_3$, 300 MHz; **C**. 2D spectrum (HSQC) of purified biosurfactant, in $CDCl_3$, 300 MHz, **D**. 2D spectrum (COSY) of purified biosurfactant, in $CDCl_3$, 300 MHz.

The results establish the SK80 product as an *sn*-1-monoolein, with the following proposed formula:

$$CH_3–(CH_2)_6–CH_2–CH=CH–CH_2–(CH_2)_4–CH_2–CH_2–COO–CH_2–CH(OH)–CH_2–OH$$

APCI MS experiments showed a molecular positive ion peak at *m/z* 357 for this main product. This value corresponds to a molar mass of 356 g/mol, which is consistent with the previous formula. In addition, several peaks in APCI MS were consistent with fragmentation schemes suggested for monoacylglycerols [10] and confirmed the presence of a long aliphatic hydrocarbon chain within the molecule. The *m/z* values reported for the positive-ion APCI mass spectrum of 1-monoolein were 357, 340, 339, 313, 283, 266, 265, 247, 135, 121, 109, and 95. The values found for SK80 were 357, 339, 283, 265, 247, 218, 205, 191, 177, 163, 149, 135, 121, 107, 89, and 75. The agreement is thought to be satisfactory when considering that impurities may be responsible for the additional peaks. Finally, the peaks at 339, 265, and 247 were attributed to fragmentations involving the glycerol moiety [10].

The commercial palm oils contain mostly triglycerides and some fatty acids. Microbial lipases have been shown to catalyze the hydrolysis of triglycerides into diglycerides, monoglycerides, glycerol, and fatty acids, and under certain conditions the reverse reaction leads to esterification and the formation of glycerides from glycerol and fatty acids [18]. In this study, lipase produced by *E. dermatitidis* SK80 hydrolyzed trioiein in a palm oil carbon source to produce monoolein biosurfactant. Monoolein is a monoglyceride that is found in the composition of human and animal bodies, such as in the complex lipid mixture of human sebum [22]. Monoglycerides are nonionic surfactants, allowing for stable emulsion, which facilitates their widespread use in a number of different fields, such as the food, cosmetic and pharmaceutical industries [7]. Interestingly, some

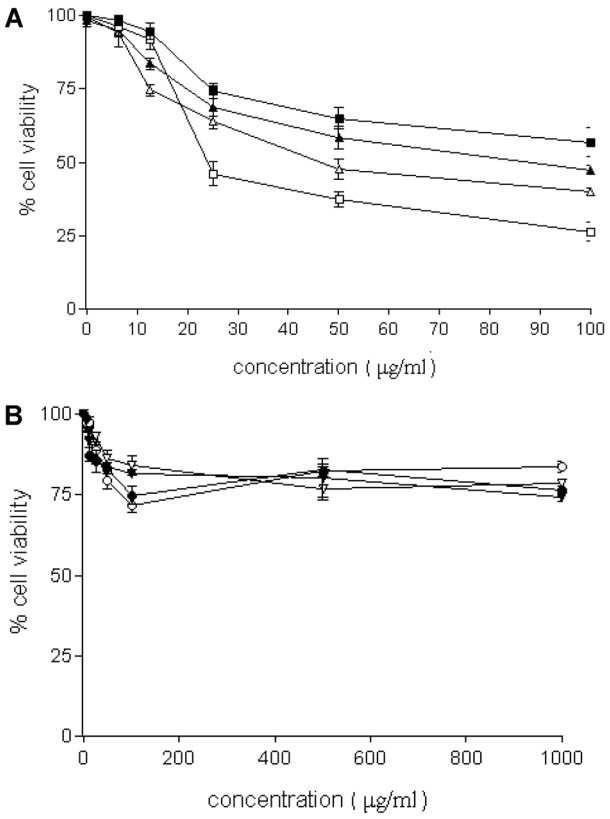

Fig. 4. Activity of the SK80 crude extract and biosurfactant against cancer.
A. Dose-dependent antiproliferative effect of SK80 crude extract and pure biosurfactant against HeLa and U937 cells after 48 h of incubation at 37°C, 5% CO_2. **B**. Dose-response curve of the SK80 crude extract and pure biosurfactant on normal cells (Vero and PBMC), concentrations varying from 0–1,000 μg/ml.

monoglycerides exhibit antiviral and antibacterial activities [3, 12, 24].

Antiproliferative Activity Against Cancer Cell Lines

We investigated the antiproliferative activity of monoolein produced by *E. dermatitidis.* The SK80 crude extract and the pure biosurfactant, monoolein, were tested for cytotoxic effects against 4 different varieties of cancer cell lines. As shown in Table 2, the antiproliferative activity was specific to the cancer cell types, those being HeLa and U937, and did not affect normal cell growth even when used at high concentrations. The most prominent antiproliferative effect was found to be against the cervical cancer (HeLa) and leukemia (U937) cell lines in a dose-dependent manner (Fig. 4A). Treatment of normal cells (Vero and PBMC) with 0.01% ethanol, as a vehicle control, and 1,000 μg/ml monoolein showed no significant antiproliferative effect (P=0.05) (Fig. 4B).

For the preliminary characterization of cytotoxicity induced by monoolein in the cancer cells tested, we first examined the changes in cell morphology induced by the treatment under a phase contrast microscope. Cell abnormalities such as cell rounding up, cell shrinkage, membrane blebbing, and loss of cell adhesion were observed in both treated cancer cells (Fig. 5A). Hoechst 33342-DNA binding dye demonstrated nuclear condensation and fragmentation into apoptotic bodies (Fig. 5B). The DNA fragmentation that occurred was confirmed by a DNA ladder assay (Fig. 6). This characteristic is commonly associated with the apoptotic process, in which the DNA is cleaved into fragments of 180 nucleosomal units by the endogenous endonuclease, caspase enzymes. Thus, it was suggested that the mode of cell death triggered by monoolein might be the process of apoptosis, which is recognized as a novel approach for anticancer drugs [11, 19]. By contrast, it was observed that no cell morphological abnormalities or DNA fragmentation took place in normal cells treated with monoolein at 1,000 μg/ml (Fig. 5), suggesting that monoolein has no toxic effects on normal cells. In light of these fascinating results, it is little wonder that the biological activity of monoolein, which specifically induces apoptotic cell death in HeLa and U937 cells, and the mechanisms of action of monoolein against these cancer lines are currently under investigation.

We isolated *Exophiala dermatitidis* capable of biosurfactant production. The production was induced by palm oil and low amounts of NH_4NO_3. The biosurfactant was purified by silica gel chromatography. The strongest surfactant-activity compound was isolated and its chemical structure elucidated by NMR and mass spectrometry, and found to be monoolein. Amongst the four cancer cell lines tested,

Table 2. IC_{50} values of the SK80 crude extract, SK80 pure biosurfactant, and commercial monoolein.

Treatment	IC_{50} (μg/ml)				
	Vero	HepG2	HeLa	U937	MCF-7
Medium control	98.16±0.34	99.28±0.76	98.21±0.14	99.23±1.03	97.29±0.67
0.05% ethanol	96.23±1.78	93.43±0.63	89.78±1.65	91.16±0.98	86.13±0.74
SK80 crude	89.11±0.76	81.58±1.65	63.24±0.79	65.23±1.24	82.16±1.23
SK80 pure	73.78±0.82	81.63±1.32	**29.89±1.36**	**49.85±1.43**	71.64±1.67
1-Monoolein	82.83±1.31	75.36±0.34	**32.67±1.32**	**48.32±1.26**	69.78±0.76

Medium control and 0.05% ethanol were used as the negative control. The lowest IC_{50} of the tested samples against cancer cell lines are represented in bold.

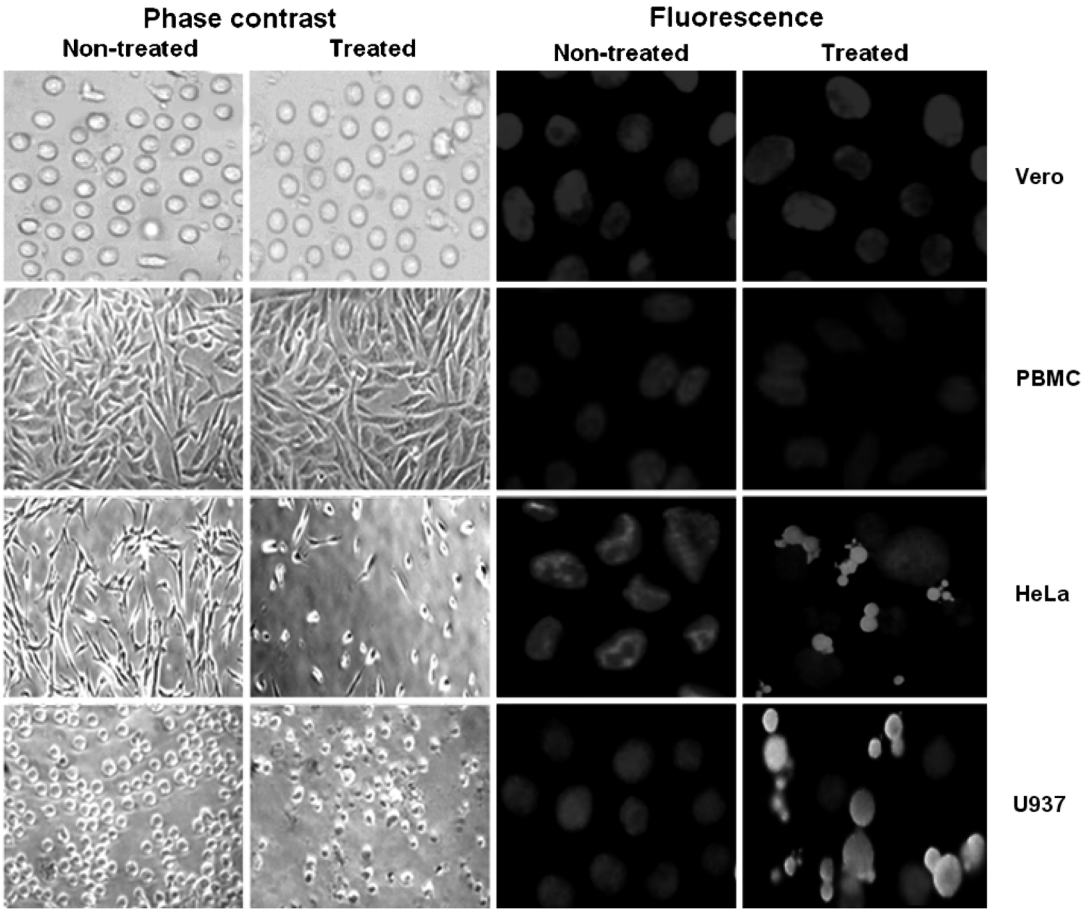

Fig. 5. Cell morphology of cancer cells (HeLa and U937) and normal cells (Vero and PBMC) treated with SK80 monoolein compared with 0.01% ethanol (vehicle control) observed under a phase contrast microscope (left panel) and a fluorescence inverted microscope (right panel) at a magnification of 400×.

monoolein showed dose-dependent cytotoxicity, specifically to the cervical cancer and leukemia cell lines. Displaying apoptosis cell death characteristics, the destruction of cells and DNA were observed for the SK80 crude extract and monoolein-treated cancer cells, but not for treated and untreated normal cells.

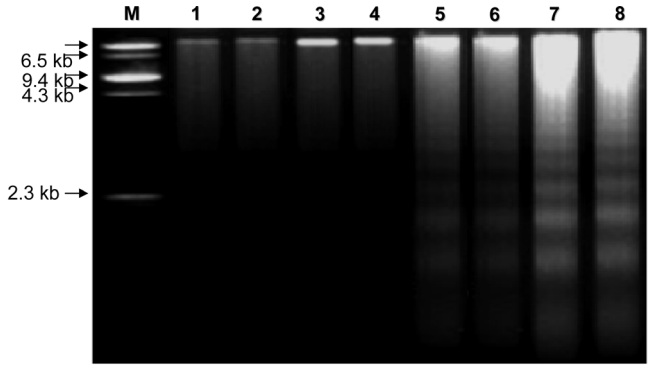

Fig. 6. Agarose gel electrophoresis of chromosomal DNA of 3×10^6 cells of cancer and normal cells treated with the SK80 crude extract or monoolein at 37°C in 5% CO_2 for 24 h.
M=molecular weight marker; lane 1, untreated HeLa cells; lane 2, untreated U937 cells; lane 3, Vero cells - SK80 extract; lane 4, Vero cells - monoolein; lane 5, HeLa cells - SK80 extract; lane 6, HeLa - monoolein; lane 7, U937 cells - SK80 extract; and lane 8, U937 - SK80 crude extract.

Acknowledgments

We would like to thank the Ministry for Higher Education of the Kingdom of Thailand for their financial support by way of the Ph.D. study of Paramaporn Chiewpattanakul. This research project was also partially supported by a Srinakharinwirot University grant.

REFERENCES

1. Altschul, S. F., T. L. Madden, A. A. Schäffer, J. Zhang, Z. Zhang, W. Miller, *et al*. 1997. Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. *Nucleic Acids Res.* **25:** 3389–3402.

2. Banat, I. M., A. Franzetti, I. Gandolfi, G. Bestetti, M. G. Martinotti, L. Fracchia, T. J. Smith, and R. Marchant. 2010. Microbial biosurfactants production, applications and future potential. *Appl. Microbiol. Biotechnol.* **87:** 427–444.
3. Bergsson, G., Ó. Steingrímsson, and H. Thormar. 2002. Bactericidal effects of fatty acids and monoglycerides on *Helicobacter pylori*. *Int. J. Antimicrob. Agents.* **20:** 258–262.
4. Bodour, A. A. and R. M. Miller-Maier. 1998. Application of modified drop-collapse technique for surfactant quantitation and screening of biosurfactant-producing microorganisms. *J. Microbiol. Methods* **32:** 273–280.
5. Cameotra, S. S. and P. Singh. 2009. Synthesis of rhamnolipid biosurfactant and mode of hexadecane uptake by *Pseudomonas* species. *Microb. Cell Fact.* **8:** 16–22.
6. Camilios, N. D., J. A. Meira, J. M. De Araújo, D. A. Mitchell, and N. Krieger. 2008. Optimization of the production of rhamnolipids by *Pseudomonas aeruginosa* UFPEDA 614 in solid-state culture. *Appl. Microbiol. Biotechnol.* **81:** 441–448.
7. Elfman-Borjesson, I. and M. Harrod. 1999. Synthesis of monoacylglycerides by glycerolysis of rapeseed oil using immobilized lipase. *J. Am. Chem. Oil Soc*. **76:** 701–707.
8. Flis, S., A. Gnyszka, I. Misiewicz-Krzeminska, and J. Splawinski. 2009. Decytabine enhances cytotoxicity induced by oxaliplatin and 5-fluorouracil in the colorectal cancer cell line Colo-205. *Cancer Cell Int*. **27:** 1–10.
9. Goh, E. M. and R. E. Timms. 1985. Determination of mono- and diglycerides in palm oil, olein and stearin. *J. Am. Oil Chem. Soc*. **62:** 730–734.
10. Holcapek, M., P. Jandera, J. Fischer, and B. Prokes. 1999. Analytical monitoring of the production of biodiesel by high-performance liquid chromatography with various detection methods. *J. Chromatogr. A* **858:** 13–31.
11. Hou, Y., M. Wu, Y. Hwang, F. Chang, Y. Wu, and C. Wu. 2009. The natural diterpenoid ovatodiolide induces cell cycle arrest and apoptosis in human oral squamous cell carcinoma Ca9-22 cells. *Life Sci*. **3:** 26–32.
12. Isaacs, C. E., H. Thormar, K. S. Kim, W. C. Heird, and H. M. Wisniewski. 1989. Antiviral and antibacterial activity of fatty acids and monoglycerides. US Patent 4997851 WO/1989/ 006124A1
13. Kitamoto, D., H. Isoda, and T. Nakahara. 2002. Functional and potential application of glycerol biosurfactants. *J. Biosci. Bioeng.* **94:** 187–201.
14. Kosaric, N., H. Y. Caoi, and R. Bhaszczyk. 1990. Biosurfactant production from *Nocardia* SFC-D. *Tenside Surfact. Det.* **27:** 294–297.
15. Patel, R. M. and A. J. Desai. 1997. Biosurfactant production by *Pseudomonas aeruginosa* GS3 from molasses. *Lett. Appl. Microbiol.* **25:** 91–94.
16. Pornsunthorntawee, O., S. Maksung, O. Huayyai, R. Rujiravanit, and S. Chavadej. 2009. Biosurfactant production by *Pseudomonas aeruginosa* SP4 using sequencing batch reactors: Effects of oil loading rate and cycle time. *Bioresour. Technol.* **100:** 812–818.
17. Rahman, P. K. and E. Gakpe. 2008. Production, characterization and applications of biosurfactants – review. *Biotechnology* **7:** 360–370.
18. Saxena, R. K., P. K. Ghosh, R. Gupta, D. W. Sheba, S. Bradoo, and R. Gulati. 1999. Microbial lipases, potential biocatalysts for the future industry. *Curr. Sci.* **77:** 101–115.
19. Shougang, J., Z. Yuangang, F. Yuejie, Z. Yu, and E. Thomas. 2008. Activation of the mitochondria-driven pathway of apoptosis in human PC-3 prostate cancer cells by a novel hydrophilic paclitaxel derivative, 7-xylosyl-10-deacetylpaclitaxel. *Int. J. Oncol.* **33:** 103–111.
20. Singh, M., V. Saini, D. K. Adhikari, J. D. Desai, and V. R. Sista. 1990. Production of bioemulsifier by SCP production strain of *Candida tropicalis* during hydrocarbon fermentation. *Biotechnol. Lett.* **12:** 743–746.
21. Sudhadham, M., S. Prakitsin, S. Sivichai, R. Chaiyarat, G. M. Dorrestein, S. B. J. Menken, and G. S. de Hoog. 2008. The neurotropic black yeast *Exophiala dermatitidis* has a possible origin in the tropical rain forest. *Stud. Mycol*. **61:** 145–155.
22. Tatara, T., T. Fuji, T. Kawase, and M. Minagawa. 1983. Quantitative determination of tri-, di-, monooleins and free oleic acid by the thin layer chromatography flame ionization detector system using internal standards and boric acid impregnated chromarod. *Lipids* **18:** 732–736.
23. Thanomsub, B., T. Watcharachaipong, K. Chotelersak, P. Arunrattiyakorn, and T. Nitoda. 2004. Monoacylglycerols: Glycolipid biosurfactants produced by a thermotolerant yeast, *Candida ishiwadae*. *J. Appl. Microbiol.* **96:** 588–592.
24. Thormar, H., G. Bergsson, and E. Gunnarsson. 1999. Hydrogels containing monocaprin have potent microbicidal activities against sexually transmitted viruses and bacteria *in vitro*. *Sex Transm. Infect.* **75:** 181–185.