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PRODUCTION AND CHARACTERIZATION OF SOPHOROLIPIDS BY *Candida bombicola* USING CATFISH FAT

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ABSTRACT

Catfish fat is a high nutrition by-product of seafood processing industry. In this work, catfish fat has been used as a sustainable and economical raw material for sophorolipids production by Candida bombicola. Sophorolipids yield was maximum as 21.8g/L after 7 days of fermentation at 25°C, pH 6, 180 rpm. The obtained sophorolipids was to contained the main component as lactonic sophorolipids, which has been confirmed by Thin layer chromatography (TLC). Sophorolipids also exhibited the ability to resistant Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa and showed the ability of free radical scavenging the dose dependent manner with IC_{50} was 4.45 mg/ml. These results suggested that sophorolipids could be used in health care products and cosmetic. Catfish fat could be used as the low cost hydrophobic carbon source to replace fatty acid for sophorolipids production.

Keywords: catfish fat by-product, sophorolipids, *Candida bombicola*, biosurfactant.

TÓM TẮT

Thu nhận và khảo sát đặc tính của sophorolipids từ quá trình lên men Candida bombicola từ mỡ cá tra

Mỡ cá tra là một phụ phẩm giàu dinh dưỡng của ngành công nghiệp chế biến thủy sản. Trong nghiên cứu này, mỡ cá tra được sử dụng như là nguyên liệu thay thế, chi phí thấp cho việc lên men thu nhận sophorolipids từ quá trình lên men chủng Candida bombicola. Kết quả cho thấy, sản lượng sophorolipids thu nhận cao nhất là 21,8g/L sau 7 ngày lên men ở 25°C, pH 6, tốc độ lắc 180 vòng/phút. Kết quả phân tích sắc kí bản mỏng cho thấy có sự hiện diện của sophorolipids dạng lactone. Sophorolipids thu nhận cũng có khả năng kháng lại một số chủng vi khuẩn như Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa và có khả năng bắt gốc tự do cao với IC_{50} = 4.45 mg/mg. Các kết quả trên cho thấy sophorolipids có tiềm năng ứng dụng trong dược – mĩ phẩm và mỡ cá tra có thể sử dụng như nguồn nguyên liệu giá rẻ cho việc sản xuất sophophorolipid.

Từ khóa: mỡ cá tra, sophorolipids, Candida bombicola, chất hoạt động bề mặt sinh học.

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1. Introduction

Biosurfactant such as sophorolipids (SLs) are surface active glycolipid compounds synthesized by some of non-pathogenic yeast species like *Candida bombiola*, *Candida apicola*, *Candida batistae*, *Candida bogoriensis*, *Wickerhamiella domericquiae* [1,2]. Among these species, *Candida bombicola* is placed at the highest of yield. In the recent years, the trend of production and use of SLs has been increasing due to their various functional properties which have advantages over the synthetic surfactant for their biodegradability, lesser toxicity, higher foaming rate, better environmental compatibility, high specific activity and selectivity over a broad range of temperatures, pH and salinity [4,6].

SLs contain O-glycosyl linkage between sophorose (2'-O-β-D-glucopyranosyl-β-D-glycopyranose) and fatty acid chain (C16-C18). There are two main structure groups of SLs, carboxyl structure (acidic sophorolipid) and ester structure (lactonic sophorolipid) [3]. At least twelve different structures of SLs have been identified that vary in acidic or latonic, degree of acetylation of sophorose unit or unsaturation in fatty acid moiety [5]. SLs have shown a variety of applications like antibacterial agents, septic shock antagonists, anticancer agents, anti-fungal and anti-viral agents, inducers of cellulase production [8, 11]. SLs have also been used as capping agents for synthesis of cobalt nanoparticle, enhancing factor in the oil recovery, stimulating agents to the metabolism of skin fibroblast cells and applications in perfume industry [7-10].

Despite possessing many commercially attractive properties and advantages over synthetic counterparts, the synthesis of SLs on a commercial scale has not been realized due to high production costs [12]. In which, the greatest contributor to production cost was the cost of raw materials, which accounted for 89% of total estimated production. In this study, we used catfish fat as the low-cost alternative to lipid substrate for SLs production [5]. In Vietnam, the catfish processing industry for export has been developing at the Mekong Delta area. However, the main component used is fish fillet, by-product such as: bone, skin, fat… have not been utilized effectively. Among them, fish fat is a by-product with high nutrition value but has not been fully utilized. The component of catfish fat contains over 70% of unsaturated fatty acid, which is suitable for using as material in SLs synthesis. This is both economically potential for low-cost SLs production and contributing to environmental protection.

2. Materials and methods

2.1. Microorganism and raw material

The SLs producing strain *Candida bombicola* ATCC 22214 was kindly gifted by Pro. Kim Eun Ki, Inha University, Korea. Sophorolipids standard 1′,4″-Sophorolactone 6′,6″-diacetate, 2,2-diphenyl-1-picrylhydrazyl (DPPH) was supplied by Sigma (USA). The

organic solvents such as n-hexane, methanol, ethyl acetate were supplied by Xilong (China). Catfish fat was procured from Agifish company, An Giang, Vietnam.

2.2. Pretreatment of catfish fat

The fish fat is cleaned by repeated water washing, then cut into small pieces and ground. After grinding, heating the fish fat indirectly at 80^{o}C, then filtrate to remove the solids. Next, the fish fat liquid was washed with 10% NaCl solution, removal of water layer to get clean fat liquid then stored at 4^{o}C until using in fermentation for SLs production (Figure 2.1).

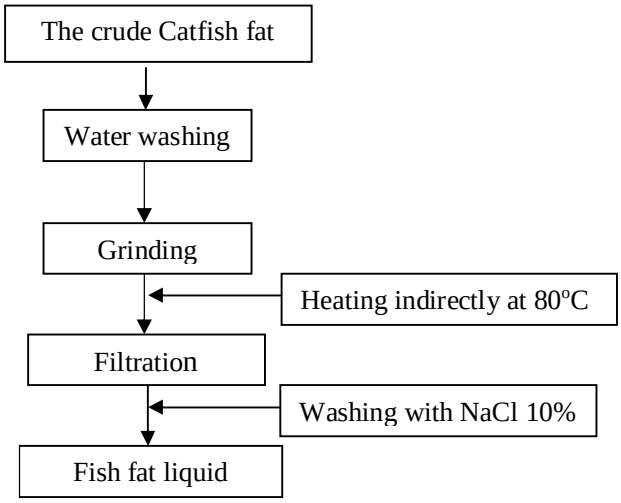

Figure 2.1. *Schematic diagram of catfish fat pretreatment*

2.3. Culture conditions and media

Candida bombicola ATCC 22214 (cryopreserved at -80^{o}C) was inoculated in MGYP media and incubated at 25^{o}C with 180 rpm for 48 hours. This culture broth was then transferred to the fermentative medium and also persevered for the further cell storage.

The 250 ml Erlenmeyer flasks containing 100 ml of fermentative medium (10% (v/v) catfish fat, 10% glucose, 0.5% yeast extract, 0.1% KH_2PO_4, 0.05% MgSO4.7H_2O, 0.01% $CaCl_2$.2H_2O, 0.01% NaCl, 0.07% peptone). Sterile fermentation medium was inoculated with 5% (v/v) seed medium to begin of SLs production at 25^{o}C, pH 6, shaking 180 rpm for 7 days.

2.4. Sophorolipids separation

The fermented broth was centrifuged at 6000 rpm, 5 minutes, collected the supernatant and extracted with n-hexane (1:1 v/v) to remove excess oil and then extracted with ethyl acetate (1:1 v/v) to obtain sophorolipids. Solution obtained after ethyl acetate extract was vacuum dried at 40^{o}C to remove the solvent.

2.5. Analysis of sophorolipids by TLC

SLs samples dissolved in ethyl acetate were spotted on Silica gel plates. The plates were then immersed in solvent systems containing chloroform/methanol/H_2O (80:10:2

v/v/v). Once the slovents front had moved approximately 2/3 the height of the plates, they were removed and sprayed with H_2SO_4 90% then dried at 100°C until spots were observed.

2.6. Antibacterial activity

The antibacterial activity was tested using agar diffusion method and determined diameter of inhibition zone on plates. To determinate the minimum inhibitory concentration, MIC method was conducted. Several bacterial strains for testing include *Escherichia coli, Staphylococus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Salmonela typhi.*

2.7. Antioxidant activity

Antioxidant activity was determined by DPPH assay. SLs sample and DPPH solution were added into 96 well plate then mixed. The plate was incubated at 37°C for 30 min and measured the absorbance at 517 nm wavelength. 96% Ethanol as negative control and 0.2 mg/ml ascorbic acid as positive control. The percentage of free radical scavenging was calculated as the formula:

% Antioxidant = [1 – (OD sample/OD control)]*100.

3. Results and discussion

3.1. Sophorolipids production

SLs production by *C. bombicola* at 25°C, pH 6, 180 rpm using glucose and catfish fat as substrate source. SLs yields at different time were shown in Figure 3.1. The results showed that SLs yield increased from the third to the fifth day and highest yield was obtained after 7 days of fermentation. The obtained SLs mixtures are typically brown oils, which are more viscous and denser than water. This result is similar to report of Cavalero and Cooper (2003) [1] and other studies by Davery *et al* (2010) [3], Solaiman *et al* (2004) [12].

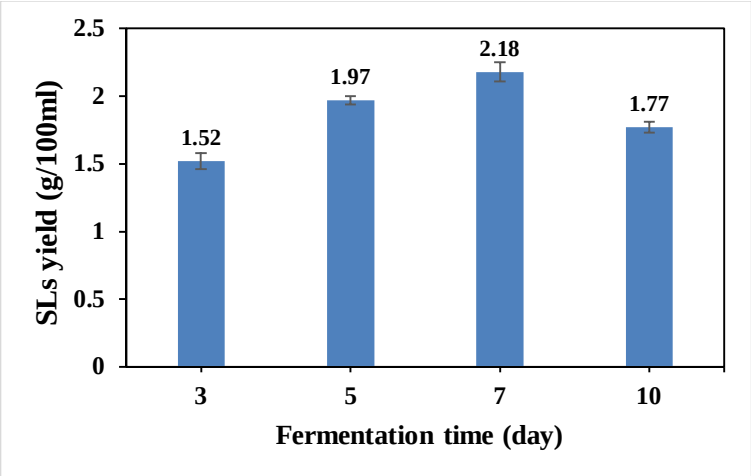

Figure 3.1. *SLs yield at different time*

3.2. Analysis of SLs composition by TLC

The fermented product was confirmed by Thin layer chromatography using 1′,4″-Sophorolactone 6′,6″-diacetate (Sigma) as the standard. The result in Figure 3.2 showed that the presence of 1′,4″-Sophorolactone 6′,6″-diacetate in fermented product and also many different forms of SLs were detected.

Figure 3.2. *Thin layer chromatography plate of sophorolipids:*
1. SLs standard (1',4"-Sophorolactone 6',6"-diacetate), 2. Synthesized SLs

3.3. Antibacterial activity of SLs

The antibacterial activity of SLs was summarized in Table 3.1 and Figure 3.4. The results showed that SLs could be against Gram positive stronger than Gram negative bacteria. Although the antibacterial activity of SLs in this study was low compared to previous studies of Shah *et al* (2007) [9] and Morya *et al* (2013) [8] , it still showed potential applications of SLs as an antiseptic, cleansing fruits and vegetables or in the combination with antibiotics to improve effective treatment.

Table 3.1. *Antibacterial activity of SLs*

	Bacterial strains	**Diameter of the inhibition zone (cm)**	**MIC (mg/ml)**
1	*Staphylococcus aureus*	2.11 ± 0.20	3.5
2	*Bacillus subtilis*	1.42 ± 0.10	5.0
3	*Escherichia coli*	1.00 ± 0.05	10.0
4	*Pseudomonas aeruginosa*	1.10 ± 0.03	10.0

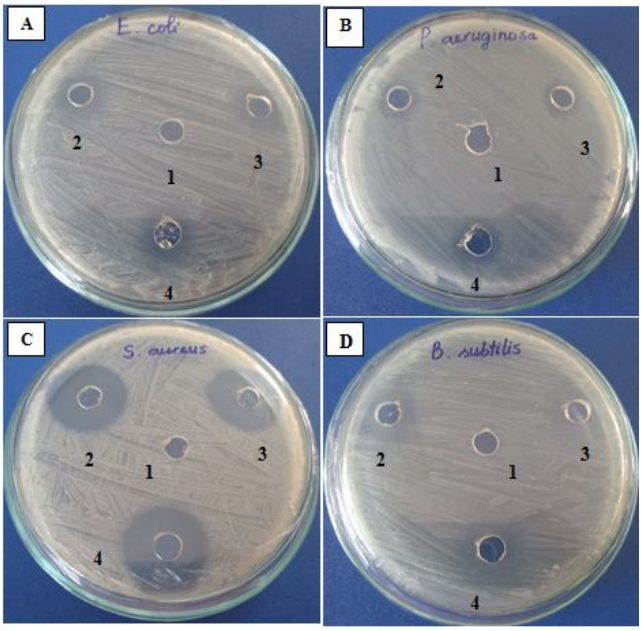

Figure 3.4. *Antibacterial activity of SLs. A: E. coli; B: P. aeruginosa; C: S. aureus; D: B. subtilis,1: negative control, 2,3,4: SLs samples*

3.4. Antioxidant activity of SLs

The ability of free radical scavenging of SLs was tested by DPPH assay (Figure 3.5). The result showed that SLs could scavenge the free radical molecules in the dose dependent manner and IC_{50} was 4.45 mg/ml. The antioxidant and antibacterial activity of SLs prove their potential applications in cosmetics and pharmaceuticals.

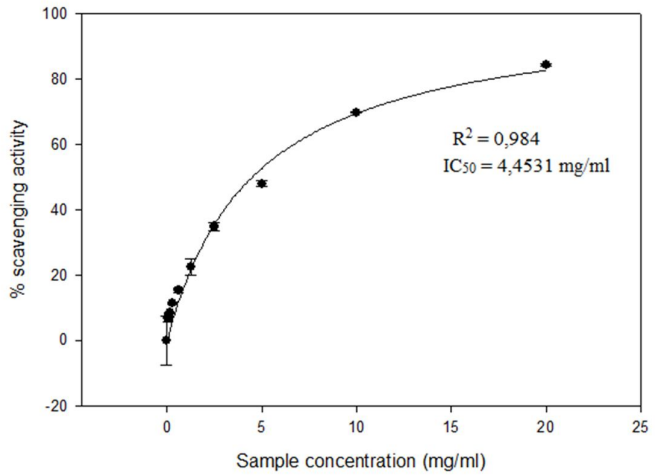

Figure 3.5. *The ability to scavenge free radical molecules of SLs.*

4. Conclusions

Sophorolipids produced by *C. bombicola* using catfish fat as substrate source alternative to costly fatty acid, the highest of SLs yield was obtained after 7 days of fermentation, 25^{o}C, pH 6. The crude SLs contained lactonic form. SLs inhibited *E. coli, S. aureus, P. aeruginosa, B. subtilis* growth and exhibited antioxidant activity with IC_{50} was 4.45 mg/ml. The present work has clearly established that fish fat can be used as a sustainable lipid source for SLs production.

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