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Production and characterization of low molecular weight sophorolipid under fed-batch culture

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HIGHLIGHTS

- The study was aimed for the production and optimization of the C12–C14 sophorolipid.
- Coconut oil (15%) and glucose (10%) were used as carbon sources.
- The maximum yield was 54 g/L, during eight day of fermentation.
- Significant surface activities, and emulsion ability were recorded.
- Molecular characterization of sophorolipid was done by LC–MS.

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ABSTRACT

The present study was designed for the production and optimization of the C12–C14 sophorolipid, using the yeast *Candida bombicola* ATCC-22214. The fermentation was carried under fed-batch culture conditions i.e., maintaining 15% coconut oil and 10% glucose as hydrophobic and hydrophilic carbon sources, respectively. A maximum yield 54.0 g/L (in 234 h) was achieved. A significant antimicrobial activity, surface activity, and emulsion ability were recorded. The native sophorolipid was found as enhancer of detergent efficacy of commercial detergent, tested on complex, smudge and oil contaminated clothes. Molecular weight of the C12 (605/623) and C14 (633/651) sophorolipids were determined by LC–MS which revealed it as diacetylated sophorolipid. This study is being important in terms of yield, which is better than the previously reported.

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1. Introduction

Sophorolipids are surface-active compounds synthesized by a selected number of yeast species such as *Candida bombicola, Candida apicola, Candida batistae, Wickerhamiella domericqiae* and *Rhodotorula bogoriensis* (Chen et al., 2006; Gorin et al., 1961; Konishi et al., 2008; Spencer et al., 1970; Tulloch and Spencer, 1968). In an estimate, the annual consumption of the sophorolipid is around 10 million ton per annum (Van Bogaert et al., 2007). Sophorolipids are mostly used as household/laundry detergents, but instead they also have some potential application in chemical, textile, food, paper, and cosmetics industries. Commercialization of sophorolipid based products is rapidly occupying the market share of the herbal cosmetics. The French company Soliance (http://www.groupesoliance.com) and the Korean MG Intobio Co. Ltd are producing sophorolipid-based cosmetics and skin health products. In addition to surfactant properties of sophorolipids, the anti-bacterial properties, especially against *Propionibacterium acnes* and *Corynebacterium xerosis*, make them a better emulsifying agent for cosmetics formulations. Sophorolipids also exhibit lower cytotoxicity. Other beneficial properties like anticancer, stimulation to dermal fibroblast metabolism, free radical scavenging skin desquamation and depigmentation, hair protection, sanitization make sophorolipid, an attractive component for cosmetic and pharmacodermatological products (Kim et al., 2002; Mager et al., 1987; Shao et al., 2012; Van Bogaert et al., 2007, 2011).

In last few years, various vegetable oils were screened for a higher production of sophorolipid with different bioactivities (Kim et al., 1997, 2002, 2005, 2009). Among numerous used substrates, the production variability was major constrain for industrial application of sophorolipid as a surfactant. The bioactivities of the macromolecule are not only class dependent but the size, structural anomaly, and the purity also contribute a great role (Morya et al., 2012). In fact, the sophorolipids synthesized by

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C. bombicola were a mixture of molecules having differences in the structural aspects like type of fatty acids part, lactonization and the acetylation patterns (Van Bogaert et al., 2007). These variations also influence the hydrophobicity of sophorolipid molecules (Tran et al., 2012; Van Bogaert et al., 2011) In nature, the Sophorolipids molecules were synthesized as a mixture of acidic and lactonic forms, and also showing a huge number of structural variability (Ashby et al., 2005; Kim et al., 2009).

Coconut oil contains mainly lauric acid ($CH_3(CH_2)_{10}COOH$) as a major fatty acid component. More than 90% of the fatty acids of coconut oil were reported as saturated fatty acids (iodine value-7–12), thus having minimum risk of oxidative damage (Gordon and Rahman, 1991). Coconut oil is one of the richest sources of medium chain fatty acids (MCFA). It was also reported that the coconut oil contains 47.5% Lauric (C12) and 18.1% Myristic (C14) acids of the total fatty acids (Gordon and Rahman, 1991).

Among the Sophorolipid producing organisms, *C. bombicola* is placed at the highest rank in terms of yield. The synchronized, de-novo and bioconversional production of sophorolipid by this organism were an additional advantage for selecting it as a producer (Kim et al., 2009). The product of the de-novo synthesis was mainly a derivative of Palmitic, Stearic, and Oleic acids (Van Bogaert et al., 2007). The bioconversion of oils for production of sophorolipids have different mechanisms then the de novo, that is glycerol of oils are being consumed by the organism as co-substrate, and the fatty acids being utilized as feedstock (Ashby et al., 2005; Kim et al., 2009). Thus, oils could serve as a feedstock as well as a nutrient source during the production of sophorolipid. A number of substrates and culture conditions were attempted and employed to achieve novel properties, and expand the applications of sophorolipid (Felse et al., 2007; Glenns and Cooper, 2006; Hu and Ju, 2001; Kim et al., 2005, 2009). All described studies were mostly carried with C18 or higher fatty acid containing substrates. The surface active properties of sophorolipid are primarily governed by fatty acid chain length, and respective modifications, i.e. position of unsaturated bond and side groups. It was reported that the long-chain length of fatty acid domain restricts the bioavailability and also diminishes the biological properties (Van Bogaert et al., 2007, 2011). Whereas the medium or small size fatty acids get easily metabolized via β-oxidation, which results in a lower yield of sophorolipid during the fermentation process. Thus, optimization of fermentation conditions to resolve the issue is yet a major challenge for the researchers (Van Bogaert et al., 2007). In previous work, various substrates for medium chain sophorolipid production were used but they are not applied to industrial processes because of the many control factors (Kim et al., 2005, 2009). In 1998, Brakemeier and coworkers used secondary alcohol (C12 and C14) as a substrate along with the glucose (carbon source) as an alternative method to produce medium chain sophorolipids (Brakemeier et al., 1998). However, this method was not adopted by the industries due to a very high cost of the secondary alcohols (Van Bogaert et al., 2011). Addressing the same issue, in the present study the coconut oil was attempted as a substrate for production of medium chain sophorolipid. Designing and establishment of fermentation conditions (pH, temperature, aeration rate, agitation, etc.) were done during study. In addition to above, surface like properties, antimicrobial properties and molecular weight were also elucidated.

2. Methods

The cryopreserved (at −70 °C) *C. bombicola* (ATCC 22214) was revived in YM broth media and incubated at 25 °C with 250 rpm for 24 h. This culture broth was then transferred to the production medium, and also persevered for the further cell storage. All chemicals were procured from Sigma–Aldrich. The culture media and components were purchased from Difco and Merck, respectively. Coconut oil and corn oil were obtained from Shindongbang Co. (Korea).

2.1. Culture conditions and media

The composition of production medium was kept similar to previous work (Kim et al., 2009), in brief, 100.0 g glucose, 5.0 g yeast extract, 1.0 g of KH_2PO_4, 0.5 g $MgSO_4{\cdot}7H_2O$, 0.1 g $CaCl_2{\cdot}2H_2O$, 0.1 g NaCl, 0.7 g peptone and 10% (w/v) vegetable oil in one liter of distilled water. The inoculum size used for this study was 5% (v/v) of total volume of the production medium. The fermentation was carried out in 30 L of fermenter (Kobiotech, Korea) on a working volume of 15 L. The culture conditions used for this study were as followed- temperature 30 °C; pH 3.5; agitation 550 rpm; aeration rate 1 vvm and duration 3–8 days. The oxygen saturation was controlled at 20% by changing the agitation speed. When necessary, the cultivation pH was adjusted by automatic/manual titration with 6 N NaOH. Samples were withdrawn from the fermentation broth at one-day intervals for further analysis.

2.2. Fatty acid composition

The fatty acid composition of oils and their respective sophorolipids were determined by gas chromatography equipped with a flame ionization detector (GC–FID, HP 6890, HP, USA) using standard method reported by Kim et al. (2009).

2.3. Optimization of operational conditions and hydrophilic & lipophilic carbon sources

Optimization of the glucose consumption rate for maximum production of sophorolipid was achieved by the two-phase batch fermentation. During the growth phase (0–72 h), the standard medium was supplemented by 10 g/L of glucose to achieve appropriate cell growth (Kim et al., 2009). After optimum cell growth, the glucose gradient was supplemented (10–200 g/L) to different cultures along with 15 g/L of coconut oil during the production phase (72–96 h). The culture conditions were as follows: agitation 250 rpm, initial pH of 5, for 25 °C temperature, flasks with working volume of 50 mL/250 mL in 4-days (three days for growth and one day for production) culture period. After that, culture broths were harvested and assayed for determination of residual oil, glucose, sophorolipid content, and estimation of dry biomass.

2.3.1. Residual oil determination and extraction of sophorolipid

The residual oil was separated from the culture filtrate by extraction method using n-Hexane (Shin et al., 2010). The top non-aqueous layer which contains vegetable oil was separated by separation funnel. The oil was recovered by evaporation of n-Hexane using rotary vacuum evaporator (Eyela, Japan), and then weighed. The aqueous phase was extracted three times by using same volume of ethyl acetate. The crude sophorolipid was collected by evaporation of the ethyl acetate using a rotary vacuum evaporator (Eyela, Japan), and then weighed.

2.3.2. Determination of residual glucose and dry cell weight

After extraction of sophorolipid and residual oil, the rest of the broth was subjected to evaporation in a rotary vacuum evaporator (Eyela, Japan), to remove the traces of solvents. This aqueous broth was centrifuged at 7000*g* for 15 min. Residual glucose was estimated from the supernatant, while the pellet was dried and weighted (Kim et al., 2009) for the biomass estimation. The glucose level was examined by Accu-Check (Loche) for feeding the deficient glucose during cultivation immediately and reconfirmed by

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