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Characterization of Biosurfactant from a Diesel-oil Degradation Bacterium and Application Potential in Beauty Care Products

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Abstract. The aim of this investigation was to analyze the characteristics of a novel biosurfactant produced from *Alcaligenes piechaudii* CC-ESB2 bacterium and then evaluate their application potential in beauty care products. In this experiment, the *Alcaligenes piechaudii* CC-ESB2 was firstly incubated in the BH medium addition of soybean oil to produce the novel biosurfactant. The biosurfactant was then extracted and purified from the culture solution for characteristic analysis, such as physical chemistry properties (i.e., conductivity, pH, viscosity, and density), surfactant activity properties (i.e. surface tension, emulsification index (E_{24}) and hydrophobicity), and critical micelle concentration (CMC). The purified novel biosurfactant was also used as an emulsifier in skin moisturizer preparation. A total of five skin moisturizers were prepared by biosurfactant and the Chinese medical herbs (i.e., Pearl powder and extracts from licorice, chamomile, aloe, and adlay) and then provided for safety (i.e., skin prick and allergic test; SPAT) and functional analysis (i.e., water retention capacity and emulsifiability). At the end of biosurfactant production, a total of 1.3g of biosurfactant powder (purity: 90.2±0.5%) was obtained. The characteristics of biosurfactant were the conductivity (85.5±0.6 ms/cm), the pH value (7.07±0.06), the viscosity (5.4±0.5 cp), the density (0.997±0.003 g/ml). The E_{24}, hydrophobicity, surface tension and CMC of 10g/L biosurfactant solution were determined to be 85.5±2.8%, 96.2±0.7%, 35.4±1.6dyne/cm and 50.5±4.3 mg/L, respectively. For skin prick and allergic test, which no show adverse effect. Concerning the results of functional analyses, the water retention capacity and emulsifiability achieved 80% in all skin moisturizers when the concentration of the biosurfactant additives > 500mg/L. The biosurfactant has the quite good characters of surface activity and the high potential for commercial applications. It may be applied in other territory in the future.

Keywords: Biosurfactant, *Alcaligenes piechaudii* CC-ESB2, Diesel-oil Degradation Bacterium, skin moisturizer.

1. Introduction

Biosurfactants are amphoteric compounds synthesized by microorganism. In general, it had lower toxicity, higher surface activity, and better thermal, salt, acid stability than traditional surfactants. At present the biosurfactin (a lipopeptide compound) produced from Bacillus subtilis ATCC-21332 are one of the good product [1].

The results of bioremediation related studies suggested that the surfactant additives can dramatically enhance the solubility of lipophilic contaminants (polycyclic aromatic hydrocarbons, PAHs) and then incerase the bioremediation rate in the treatment of oil contaminated soil [2]. Unfourtantly, the traditional surfactants has often accumulated in the environment. Therefore, the surfactants decomposing microorganism was investigated comprehensively. In this topic, the biodegradation of linear alkylbenzene sulfonate (LAS; the most widely used anionic surfactants in the world) is popularly in worldwide. At present the strains of LAS decomposing microorganism had been separated from sewage that include *Pseudomonas, Aquaspirillum, Aeromonas and Alcaligenes* [3-4]. Additionally, the biodegradation of surfactants and polychlorinated biphenyl (PCB) simultaneously was achieved recently by single strain of genetically

modified bacterium. Even the problem of pollution and toxicity of the surfactants has been resolved partially, it is still a great damage for the environment. Therefore the scientists have high regard for biosurfactants because of their biodegradability, high surface activity and low toxicity.

The objective of this investigation is to analyze the characteristics of the novel biosurfactant produced from *Alcaligenes piechaudii* CC-ESB2 bacterium and evaluate the application potential on replacing chemically synthesized surfactants for development of green beauty care products. The first stage of this study is to produce the biosurfactant by *Alcaligenes piechaudii* CC-ESB2 incubation. The biosurfactant was then extracted and purified by novel green extraction method. In the third stage, the physical chemistry properties (i.e., conductivity, pH, viscosity, and density), surfactant activity properties (i.e. surface tension, emulsification index (E_{24}) and hydrophobicity), and critical micelle concentration (CMC) of novel biosurfactant were examined. The purified biosurfactant was then used as an emulsifier in the preparation of skin moisturizer for safety (i.e., skin prick allergic test; SPAT) and functional analyses (i.e., water retention capacity and emulsifiability).

2. Materials and Methods

In the results of previous investigations related to biosurfactant, Chang [1] showed that the surface tension was reduced to 30 dyne/cm after eight hour of incubation of *Bacillus subtilis* (ATCC21332) by two-stage culture with cell immobilization technology in semi-continuous culture system. Hori [5] found when 10-carbon decanoate used as the carbon source had a higher biosurfactant production rate than 6-carbon glucose. The yield of rhamnolipid (one kind of biosurfactant) will also be affected by the concentration of nitrogen, phosphorous, magnesium, iron, manganese and salinity, acidity, temperature, oscillation frequency, C:N ratio [5]. Additionally, when the nitrogen source was altered, the structure of biosurfactant will also be changed [5].

In summary, the factors associated with biosurfactant production include acidity, temperature, source of carbon, concentrations of nitrogen, phosphorous and inorganic salt (i.e., magnesium, iron, manganese and calcium). The optimal medium applied in this investigation to produce the biosurfactant had been identified in our previous investigation [6]. The details of methods are shown below:

2.1. Pure strain culture

The *Alcaligenes piechaudii* CC-ESB2 was used in this investigation, which is a diesel-oil biodegrading bacterium isolated from oil-contaminated soil. In the first step, the *A. piechaudii* CC-ESB2 was incubated in 500ml of BH medium with 0.2% of soybean oil in conical flask. The bacterium was cultivated for 5 days in a shaking incubator (160rpm) at 30℃ to produce the novel biosurfactant. The BH medium consists of K_2HPO_4(1g/L), KH_2PO_4(1g/L), NH_4NO_3(1g/L), $MgSO_4$-$7H_2O$(0.2g/L), $CaCl_2$-$2H_2O$(0.02g/L) and $FeCl_3$(0.05g/L).

2.2. Biosurfactant extraction and purification [7-8]

After 5 days of incubation, the soybean oil layer and thalli in conical flask were removed firstly. The culture solution was then extracted twice with equal volume of ethyl acetate. The extracted ethyl acetate solution was then concentrated by rotary vacuum evaporator (PANCHUM R-2000V) to produce the concentrated liquid biosurfactant solution. The n-hexane was used to remove the residual olein in the concentrated liquid biosurfactant solution and then concentrated again. The biosurfactant powder was obtained by freeze drying (Freezer Deyer- FD-series, PANCHUM) of the concentrated liquid biosurfactant solution.

2.3. Characteristics analysis

The biosurfactant solution was prepared by adding 1.0g of biosurfactant powder in 100mL of deionized water (Concentration: 10g/L) for assays of physical chemistry properties, surfactant properties and emulsification property. The acidity, conductivity, density and viscosity were detected by pH meter, conductivity meter, densitometer and viscometer respectively. The surface tension was detected by surface tension meter (KRuSS, Germany) with ring method [9-10]. For emulsification index examination, the culture solution was to perform 30mins of centrifugalization in 10000 rpm. The supernatant was then mixed with

soybean oil with ratio 2:3 and then in 2min vibration. After 24 hours of settlement, the solution will divided into three layers: water layer, emulsion layer and oil layer. The emulsificaton index was then calculated by equation 1[11].

For hydrophobicity [12], 100 mL of biosurfactant solution(concentration:1g/L) was prepared and then mixed with 0.5% of soybean oil. After 2mins of vibration, the solution was settled in 15mins. The water layer was then extracted for hydrophobicity examination.

The equation for hydrophobicity calculation is shown in below (equation 2):

Emulsificaton index (E_{24},%) = Height of emulsion layer / Total height of solution) ×100%.....(equation 1)

Hydrophobicity = 100 % × OD400 of water layer / OD400 of biosurfactant solution……..(equation 2)

The CMC was detected according to the method described by Mitra and Dungan(2002) [13]. Firstly, several different concentration of biosurfactant solution (10、30、50、80、100、120、150 and 200 mg/L) (diluted from the 10g/L biosurfactant solution) was prepared and the surface tension was examined. The CMC was defined as the turning point of concentration-surface tension curve.

2.4. Skin moisturizers preparation for safety and functional analysis

The several different concentration of biosurfactant solution (100、200、300、400、500 mg/L) was prepared by 90.2% pure of biosurfactant powder. The biosurfactant solutions was then mixed with pearl powder solution (10g/100mL), extracts of licorice, chamomile, aloe, and adlay respectively in the ratio 1:10(v/v) to produce skin moisturizers. The emulsifiability of each skin moisturizers was detected after 24 hours of settlement. For examination of water retention capacity, 0.2 mL of skin moisturizers was daubed on the skin with 5cm of diameter at 24°C of cooling room. The water retention capacity was detected after 20mins of overlay on the skin by moisture titrator. The SPAT was performed by the method described in literatures [14-17].

3. Results and Discussion

3.1. The yield on Biosurfactant

At the end of incubation, 10.2g of concentrated liquid biosurfactant solution was extracted from 1L of culture solution. A total of 1.3g of biosurfactant powder (purity: 90.2±0.5%) was obtained after purification and freeze drying of the 10.2g of concentrated liquid biosurfactant solution.

3.2. Characteristics of the novel biosurfactant

The pH value, EC value, density, viscosity, emulsificaton index and hydrophobicity were 7.07±0.06, 85.5±0.6 μs/cm, 0.997±0.003 g/mL 5.4±0.5 cP, 85.5±2.8 % and 96.2±0.7% respectively in biosurfactant solution at concentration of 10g/L. Additionally, the emulsificaton index and hydrophobicity were increased accompany with the increase of solution concentration. For surface tension analysis, the surface tension of deionized water was decreased from 72 dyne/cm to 35.4±1.6 dyne/cm when novel biosurfactant added in deionized water (Concentration: 10g/L). The surface tension was decreased remarkably than the result shown in 2006(47.2~55.6 dyne/cm) [18].

The several different concentration of biosurfactant solution was prepared for CMC assays. The surface tension in different concentration of biosurfactant solutions were shown in Table 1. The CMC (the turning point of concentration-surface tension curve) was about 50 mg/L (Figure 1).

Table 1. The surface tension in different concentration of biosurfactant solutions

Biosurfactant Conc. (mg/L)	0	10	30	50	80	120	150	200
Surface tension(mN/cm)	72.5	65.8	56.3	50.5	48.8	48.2	47.8	48.3

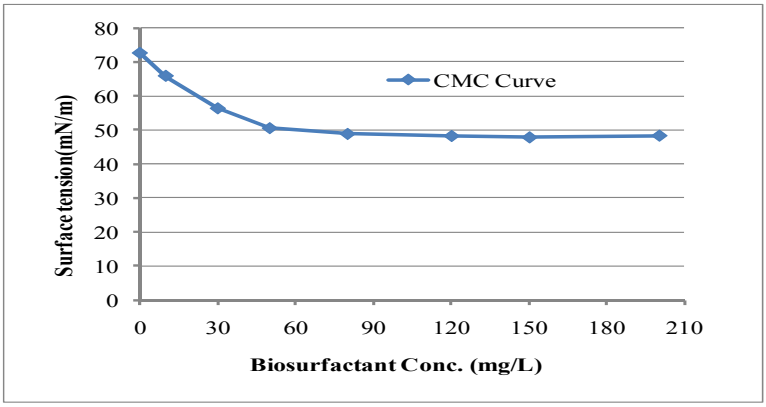

Figure 1. The concentration-surface tension profile of biosurfactant solution

According to the above mentioned, the characteristics of biosurfactant produced from *Alcaligenes piechaudii* CC-ESB2 were summarized in Table 2.

Table 2. The characteristics of biosurfactant solution produced from Alcaligenes piechaudii CC-ESB2.

Conductivity (ms/cm)	pH	Density (g/mL)	Viscosity (cp)	Emulsification index (E_{24}) (%)	Hydrophobicity (%)	Surface tension (dyne/cm)	CMC (mg/L)
55.5±0. 6	7.07±0.06	0.997± 0.003	5.4±0.5	85.5±2.8	96.2±0.7	35.4±1.6	50.5±4.3

3.3. The application potential of the novel biosurfactant in skin moisturizer

The water retention capacity and emulsifiability of the novel biosurfactant in skin moisturizers were shown in Figure 2 and 3. The water retention capacity and emulsifiability were increased accompany with the increase of the biosurfactant concentration. The water retention capacity and emulsifiability achieved 80% in all skin moisturizers when the concentration of biosurfactant additives > 500mg/L.

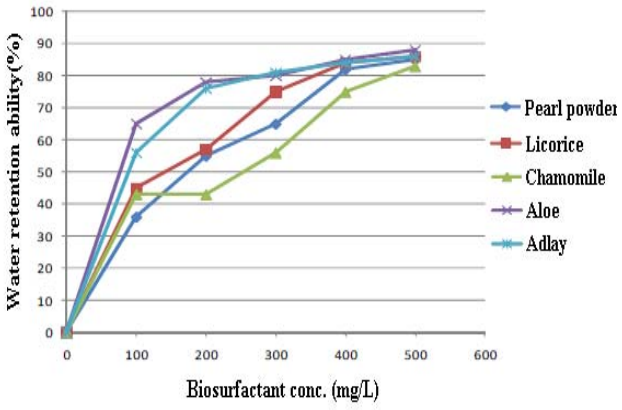

Figure 2. The water retention capacity of biosurfactant in skin moisturizers.

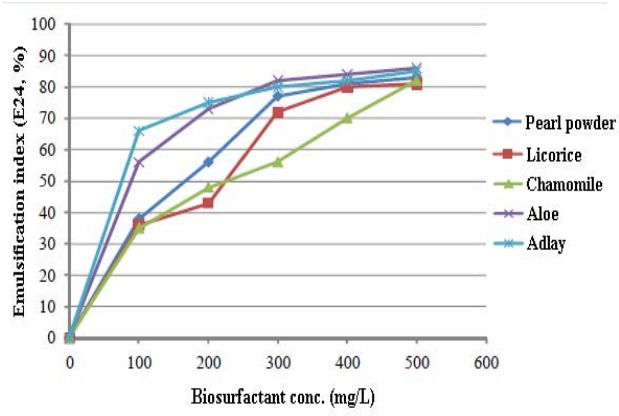

Figure 3. The emulsifiability of biosurfactant in skin moisturizers.

For SPAT, a total of 30 volunteers participants in this investigation. There had no been any adverse effects observed in this test.

4. Conclusion

The *Alcaligenes piechaudii* CC-ESB2 bacterium was used to produce a novel biosurfactant. The properties of biosurfactant and application potential on skin moisturizers were investigated. The novel

biosurfactant had excellent properties (water retention capacity and emulsifiability) for development of skin care products. It may be applied in other territories in the future.

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