Structure and characterization of a biosurfactant produced by *Pseudomonas aeruginosa* strain KO9

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Abstract—*Pseudomonas aeruginosa* strain KO9, isolated from petroleum-contaminated soil in Karamay oil field (China) was used to produce biosurfactant. The biosurfactant could reduce surface tension to 28 mN/m and emulsified diesel up to E₂₄ of 81% with a critical micelle concentration of approximately 85.82 mg/L. The dried biosurfactant was light yellow powder, and the yield was 1.0866 g/L. Fourier transform infrared (FT-IR) spectrum of extracted biosurfactant indicates the key component is glycolipid primarily consisting of lipid and carbohydrate. The crude biosurfactant showed comparable stability over a wide range of pH, temperatures and high salt concentrations, as compared to the two commercial surfactants, sodium dodecyl sulfate (SDS) and cetyltrimethyl ammonium bromide (CTAB). It has potential for use in a variety of biotechnological and industrial applications.

Keywords—*Pseudomonas aeruginosa*; Biosurfactants; Glycolipid; Surface tension; Emulsification

I. INTRODUCTION

Surfactants can be classified into two main groups: synthetic surfactants and biosurfactants. Biosurfactants are amphiphilic compounds with pronounced surface and emulsifying activities produced by microorganisms, such as bacteria, fungi, and yeast^[1, 2]. When compared to synthetic surfactants, biosurfactants have several advantages, including high biodegradability, low toxicity, low irritancy, and compatibility with human skin^[3, 4]. Therefore, biosurfactants appear preferable for the environmental applications and have a potential use in medical, cosmetic, and food industries, etc^[5].

Biosurfactants comprise a diverse group of surface-active molecules which include glycolipids, lipopeptides, polysaccharide-protein complexes, protein-like substances, lipopolysaccharides, phospholipids, fatty acids and neutral lipids^[1, 6]. Glycolipids are the most common biosurfactants that have been isolated and studied among these groups. It is mainly produced by *Pseudomonas aeruginosa* strains.

In this study, *Pseudomonas aeruginosa* KO9, isolated from petroleum-contaminated soil in Karamay oil field (China), was used to produce a biosurfactant. The chemical structures of the components in the crude biosurfactant were preliminarily determined by using Fourier transform infrared (FT-IR) spectroscopy, and their physicochemical properties were investigated in a comparison with those of the synthetic surfactants, including SDS and CTAB.

II. MATERIALS AND METHODS

A. Bacterial strain and culture growth conditions

P. aeruginosa strain KO9 was isolated from petroleum-contaminated soil in Karamay oil field. The isolated strain was maintained on nutrient agar slants at 30 °C and was subcultured every 2 weeks. The medium used to grow *P. aeruginosa* KO9 strain was a mineral salts (MS) medium consisting of CaCl₂ (7 μM), MgSO₄·7H₂O (800 μM), NH₄NO₃ (50 mM), KH₂PO₄ (30 mM), Na₂HPO₄ (40 mM), and FeSO₄·7H₂O (2 μM). A glucose mineral salts (GMS) medium is defined as glucose (5%)-supplemented MS medium. The pH of GMS medium was initially adjusted to 7.0 by 1.0 M HCl.

B. Biosurfactant Extraction

The biosurfactant produced by strain KO9 were extracted following O. Pornsunthorntawe et al. (2008)^[7].

C. Preliminary characterization of biosurfactant

The chemical structures of the components in the crude biosurfactant sample were preliminarily determined by using Fourier transform infrared (FT-IR) spectroscopy^[8].

D. Surface tension assay

The surface tension (ST) measurement of aqueous solution at different surfactant concentrations was determined in a Surface Tensiometer (Model CBVP-A3, FACE, Japan), using the Plate method. The critical micelle concentration (CMC) was determined from the break point of the surface tension versus its log of bulk concentration curve. All measurements were carried out at 25±1 °C and repeated three times, then average values were obtained.

E. Emulsion test assay

A mixture of 4ml supernatant and 6ml diesel was vortexed for 2 min and the height of emulsion layer was measured after 24 h to determine the emulsion index (E₂₄)^[9].

$$E_{24} (\%) = \frac{\text{The height of emulsion layer}}{\text{The height of total solution}} \times 100 \quad (1)$$

F. Stability testing

The crude biosurfactant sample, sodium dodecyl sulfate (SDS) and cetyltrimethyl ammonium bromide (CTAB) were

prepared at the CMCs for stability test. The prepared surfactant solutions were incubated in a 90 °C water bath at different time intervals before cooling to room temperature. The pH stability was investigated by adjusting the solutions to different pH values. The effect of salinity on the activity of the surfactants was investigated by adding NaCl of different concentrations. The surface tension and emulsion index were measured and used to indicate the stability for thermal, pH and salinity stability testing.

III. RESULTS AND DISCUSSIONS

A. Physicochemical properties

The dependence of surface tension on the biosurfactant concentration studied is shown in Fig. 1. Surface tension decreased rapidly from 75.2 to 28.4 mN/m with increases in the biosurfactant concentration up to 108.67 mg/L. Further increases in the biosurfactant concentration, only slowly reduced the surface tension from 28.4 to 26.6 mN/m. The CMC value was determined to be 85.82 mg/L, from the intersection of regression lines that describe two parts of the curve.

Figure1 Effect of biosurfactant concentration on surface tension

Besides, Table I summarizes the physicochemical properties of different biosurfactant producing microorganisms reported in the literature. Compared to the available literature, the CMC value of biosurfactant produced by *Pseudomonas aeruginosa* KO9 on glucose as the carbon source (CMC = 85.82 mg/L) is the lowest and it has better emulsification ability able to achieve an E_{24} of 81% for diesel. This indicates strong surfactant activity comparable to that of other biosurfactants.

B. Preliminary characterization of biosurfactant

5% (v/v) *P. aeruginosa* KO9 (10^7 cells/mL) was inoculated in 100 mL GMS medium at a temperature of 30°C and an agitation rate of 180 rpm. After 3 days cultured, biosurfactant was extracted, purified and freeze-dried, light yellow powder was obtained, and the yield was 1.0866 g/L.

Fig. 2 shows FT-IR spectrum profile of dried biosurfactant produced by *P. aeruginosa* KO9 grown on 5% (w/v) GMS medium. Preliminary analysis revealed that the biosurfactant produced by *P. aeruginosa* KO9 might be glycolipid primarily consisting of lipid and carbohydrate.

There are many reports on the isolation and surfactant production of different species of the genus *Pseudomonas*. However, the surfactants produced by *P. aeruginosa* has been reported to produce more often lipid-carbohydrate compounds specially rhamnolipid-type biosurfactants. FT-IR spectrum of the KO9 biosurfactant disclosed a broad stretching peak at 3398 cm^{-1} , characteristic of O-H stretching vibrations of hydroxyl groups. The strong adsorption around 2928 cm^{-1} is expected to be the symmetric stretch (ν C-H) of CH_2 and CH_3 groups of aliphatic chains. The corresponding symmetric stretch is seen at 2856 cm^{-1} . Also, an characteristic peak displayed at 1738 cm^{-1} and a weak symmetric stretching peak around 1465 cm^{-1} indicate the presence of ester carbonyl groups (C=O in COOH) in the biosurfactant while the C-O stretching bands at $1300\text{--}1000\text{ cm}^{-1}$ confirm the presence of the bonds formed between carbon atoms and hydroxyl groups in the chemical structures of the rhamnose rings. The absorption peak around 1050 cm^{-1} can be related to the presence of polysaccharide or polysaccharide-like substances in the biosurfactant.

TABLE I. COMPARISONS OF PHYSICOCHEMICAL PROPERTIES OF DIFFERENT BIOSURFACTANTS

Microorganism	Minimum ST (mN/m)	E_{24} (%)	CMC (mg/L)	Reference
<i>Lactobacillus paracasei</i>	41.8	Data not shown	2500	[10]
<i>Rhodococcus erythropolis</i> 16 LM.USTHB	31.9	63	287	[11]
<i>Pseudomonas fluorescens</i>	32.0	Data not shown	290	[12]
<i>Flavobacterium</i> sp. MTN11	26.0	Data not shown	300	[13]
<i>Pseudomonas aeruginosa</i> SP4	29.0	Data not shown	200	[7]
<i>Pseudomonas aeruginosa</i> KO9	26.6	81	85.82	Present article

Figure 2 FT-IR spectrum profile of dried biosurfactant produced by *P. aeruginosa* KO9 grown on GMS medium

C. Effect of temperature, pH and salinity on biosurfactant stability

Fig. 3a shows the effect of temperature on the surface tension and E_{24} of the biosurfactant produced from *P. aeruginosa* KO9 as compared to the two synthetic commercial surfactants, SDS and CTAB. The results show that the three tested surfactants were able to withstand 90 °C up to 2 h without a reduction in surface tension. However, emulsification capacity of biosurfactant was more stable than SDS and CTAB. In addition, the heat tolerance of the tested surfactants was studied by autoclaving at 121 °C for 15 min. For both the biosurfactant and CTAB, the surface tension and emulsion index remained unchanged, indicating that both surfactants possess excellent thermostable. In the case of SDS, the ST increased remarkably from 29.82 mN/m to 36.75 mN/m and the E_{24} decreased from 70.2% to 51.8% after the heat treatment. The result suggested that deterioration of SDS occurred at a temperature of 121 °C.

Fig. 3b shows the effect of pH on ST and E_{24} in the presence of the biosurfactant, as compared to the two commercial surfactants. As can be seen in Fig. 3b, both SDS and CTAB were more stable throughout the studied range of pH. In contrast, the activity of biosurfactant and its emulsification ability was affected by the pH. When the pH was under 5, the biosurfactant activities were above 30 mN/m. Correspondingly, the emulsification ability was limited to the acid pH and emulsification indexes were less than 55%. However, both biosurfactant activity and emulsification capacity was relatively stable between pH 6 and pH 11.

Fig. 3c demonstrates the effect of addition of NaCl on the surface tension and emulsification capacity of the surfactants. As it is shown, negligible changes were occurred on the two commercial surfactants with an increase of NaCl concentration, whereas the surface tension and emulsification capacity of biosurfactant remained constant throughout the studied range of salinity.

When compared to the commercial surfactants, the biosurfactant possessed superior properties in both heat and salinity stabilities, and it was quite constant between pH 6 and pH 11. These findings suggest that the robust characteristics of the crude biosurfactant are suitable for applications under extreme conditions, such as in microbial enhanced oil

recovery (MEOR) and in the bioremediation of a polluted environment. Moreover, this biosurfactant has great potential for biomedical applications due to its thermostability even after being submitted to autoclave sterilization.

Figure 3 Stability of the biosurfactant as compared with SDS and CTAB. (a) ST and E_{24} of surfactants after heat treatment at 90 °C with different heating times. (b) Effect of pH on surface activity of surfactants. (c) Effect of salinity on surface activity of surfactants.

IV. CONCLUSIONS

In this study, the crude biosurfactant produced from *P. aeruginosa* KO9 was chemically characterized as glycolipid mainly consisting of lipid and carbohydrate. It was able to reduce the surface tension of pure water to 26.6 mN/m and achieve the E₂₄ of 81% for diesel, besides the CMC was found to be 85.82 mg/L. When compared to synthetic surfactants, including sodium dodecyl sulfate (SDS) and cetyltrimethyl ammonium bromide (CTAB), the crude biosurfactant showed excellent stability over a wide range of pH and temperatures and high salt concentrations which suggest that it is suitable for use in oil fields, biomedical applications and removal of oil pollutions.

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