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Characteristics of Biosurfactant Produced by *Pseudomonas* sp. EL-G527 from Activated Sludge

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Pseudomonas sp. EL-G527 was grown to produce a biosurfactant on 2% *n*-hexadecane as the energy and carbon source. This biosurfactant significantly reduced the surface tension of water from 72 to 28 dyne/cm at a critical micelle concentration(CMC) of 140 mg/l at pH 2.0. As the pH value decreased, the reduction in the surface tension due to the biosurfactant increased. The surface activity of the biosurfactant was unaffected when the NaCl concentration was increased to 5% and the calcium ion concentration increased to 100 mM, plus it remained stable at 100℃ for 180 min.

Key words : Biosurfactant, surface tension, emulsification, *Pseudomonas* sp.

1. Introduction

Biosurfactants are microbially produced compounds that exhibit surface activity. Biosurfactants are amphiphilic molecules consisting of hydrophobic and hydrophilic domains[1]. Due to their amphiphatic nature, biosurfactants can preferentially partition at an interface between different fluid phases, such as oil/water or water/air. As such, since this characteristic can confer excellent detergency, emulsifying, foaming, and dispersing traits[2,3]. Currently, chemically synthesized surfactants are widely used in the petroleum, pharmaceutical, cosmetic, and food industries. However, due to the high production costs and health hazards related to synthetic surfactants, plus increasing consumer demand for natural products, the development of biosurfactants has become increasingly important[4,5].

Microorganisms produce a variety of biosurfactants[6], some of which have attracted considerable interest in recent years due to their low toxicity, effectiveness under extreme conditions, and potential role in saving the natural ecosystem, for example, microbial-enhanced oil recovery during oil spills[7]. As biosurfactants are readily biodegradable and can be produced from renewable and cheaper resources, they may eventually be able to replace synthetic surfactants[1].

Despite the many reports on microorganisms producing biosurfactants or bioemulsifiers, reports of microorganisms secreting physicochemically stable biosurfactants are rare. Accordingly, this study describes the physical and chemical properties of the biosurfactant produced by *Pseudomonas* sp. EL-G527.

2. Materials and Methods

2.1. Microorganism and Culture Conditions

The microorganism used in this study was *Pseudomonas* sp. EL-G527, which was isolated from activated sludge samples. A mineral salt medium containing the following components (g/l) was used to cultivate the bacterial strain; KH_2PO_4 3.0, K_2HPO_4 3.0, $MgSO_4 \cdot 7H_2O$ 0.2, NH_4NO_3 2.0, $CaCl_2 \cdot 2H_2O$ 0.025, and $FeSO_4 \cdot 7H_2O$ 0.015. The pH of the medium was adjusted to 7.0 and sterilized using an autoclave. The *Pseudomonas* sp. EL-G527 was grown for 4 days in the mineral salt medium containing 2%(w/v) *n*-hexadecane as the sole

carbon source at 30℃ and 200 rpm. Cells were removed from the culture by centrifugation at 12,000×*g* for 15 min. The culture supernatant was then used for measuring the surface tension and emulsification activity.

2.2. Biosurfactant Extraction

Bacterial cells were removed from the biosurfactant-containing medium by centrifugation (12,000×*g*, 20 min, 4℃) and filtration(Whatmann No. 1). The pH of the supernatant was adjusted to 2.0±0.5 with 1N HCl for biosurfactant solubility reduction. The biosurfactant was extracted with an equal volume of ethylacetate. The solvent was then evaporated and the residue washed with 3 volumes of *n*-hexane to remove any hexadecane. The crude material thus obtained was dissolved in distilled water and freeze dried.

2.3. Properties of Biosurfactant

The effect of pH on the stability of the biosurfactant was tested based on incubation in solutions with different pHs for 24 h. As such, the biosurfactant solution(0.1%) was dissolved in 10 ml of various buffer solutions(pH 2-10) separately. The thermostabilities of the biosurfactant were measured after preincubation for 180 min at 100℃ before the biosurfactant assay. The effects of various concentrations of sodium chloride (0-5.0%) and calcium chloride(10-100 mM) were also determined by adding sodium chloride and calcium chloride to the assay mixture.

2.4. Analytical Methods

The surface tension between the culture supernatant and crude biosurfactant was determined using a Tensiometer(Fisher Scientific, Surface Tensiomat®21). The biosurfactant concentration was estimated by determining the culture supernatant factor necessary to reach the critical micelle concentration(CMC)[8]. If the biosurfactant concentration falls below the CMC, the surface activity will then only depend on the concentration of the surface active molecules, thereby resulting in an increased surface tension value. The dilution factor(Fcmc) is thus a direct measure of the biosurfactant concentration. To measure the emulsifying activity, test samples(0.2 ml) were introduced into a 50-ml flask containing distilled water to a final volume of 7.5 ml. Next, 0.1 ml of hexadecane was added, then the samples were vortexed at a high speed for 1 min and the resulting emulsion allowed to stand for 10min. Its absorbance was measured at 540 nm using a spectrophotometer[9].

3. Results and Discussion

The biosurfactant was found to be soluble in water, methanol and ethanol, yet not in benzene, *n*-hexane, ethylether, or dichloromethane(data not shown).

A plot of the surface tension relative to the crude biosurfactant concentration is shown in Fig. 1. The biosurfactant exhibited excellent surface tension-reducing activities. The surface tension of water decreased from 72 to 28 dyne/cm with an increasing biosurfactant concentration up to 140 mg/l at pH 2. When the solution pH changed, the CMC also changed. At pH 2, the CMC was about 140 mg/l, whereas at pH 8-10 it was about 370 mg/l.

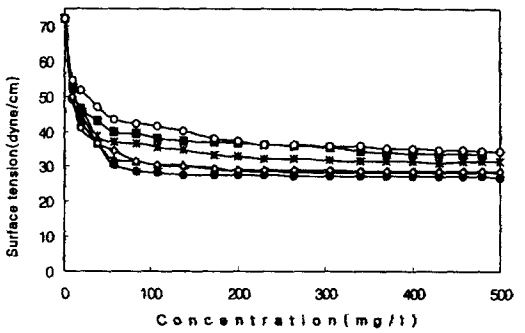

Fig. 1. Surface tension versus concentration of biosurfactant added to each buffer. Symbols : -●-, pH 2 ; -▲-, pH 4 ; -◇-, pH 6 ; -*-, pH 7 ; -■-, pH 8 ; -○-, pH 10.

Fig. 2 shows the effect of pH on the emulsifying activity. The emulsifying activity was decreased 84% at pH 6~10, whereas it remained stable at pH 4. The effect of pH on the surface tension reducing activity has already been reported for biosurfactants from different microorganisms, for example, the maximum activity of liposan from *Candida lipolytica* is between pH 2.0 and 5.0[10], whereas for emulsan, a sharp maximum activity

is obtained between pH 5.0 and pH 6.0 with no activity above pH 7.0[11]. Chemically synthesized surfactants, such as sodium dodecyl sulfate(SDS) and linear alkylbenzene sulfonate(LAS), exhibit a stable surface tension from pH 4.0 to 9.5, yet an increased surface tension from 27 to 32 dyne/cm at pH 10.3[12]. In addition to surface tension, the stabilization of an oil and water emulsion is commonly used as a surface activity indicator[13]. Table 1 presents the emulsifying activity of the biosurfactant with various oils and hydrocarbons. The biosurfactant exhibited a high emulsification activity with all the compounds tested, except for paraffin oil, plus a high emulsion stability. This result is similar to that of Kaplan and Rosenberg[11].

Fig. 3 shows the effect of heat treatment on the biosurfactant activity of the *Pseudomonas* sp. EL-G527 culture. No appreciable change in the biosurfactant properties occurred when the culture broth suspended in various pH solutions was heated. That is, the biosurfactant was found to be stable at 100℃ for 180 min for the entire pH range tested. There was also no change in the biosurfactant activity at 121℃ for 15 min. Liposan has only been reported to be stable up to 70℃[10].

Sodium salt, a major component of sea water, and calcium salt, included in industrial water, frequently break the emulsion between oil and water in a practical process[12,14]. Accordingly, the effect of NaCl and $CaCl_2$ concentrations on the stability of the surface tension reducing activity

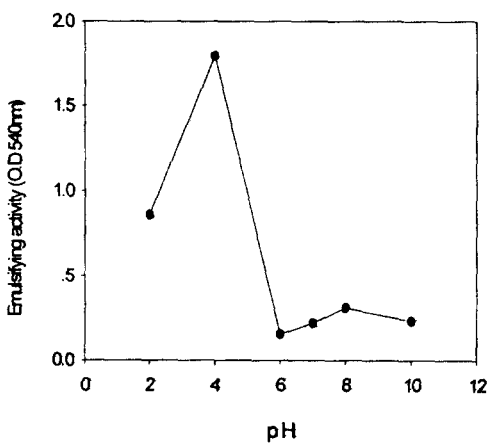

Fig. 2. Effect of pH on emulsifying activity of the biosurfactant.

Table 1. Emulsifying activity and stabilization of emulsions by biosurfactant

Substrates	Emulsifying activity	Decay constant (K_d, 10^{-3})*
n-Dodecane	2.354	-0.700
n-Tetradecane	2.614	-0.200
n-Hexadecane	2.037	-0.200
n-Octadecane	2.504	-0.050
Paraffin	0.147	-3.000
Olive oil	2.614	-0.020
Soybean oil	2.066	-0.100
Corn oil	2.070	-0.200
Peanut oil	2.071	-0.100
Caster oil	2.235	-0.043
Crude oil	3.104	-0.100
2-Methylnaphthalene	2.974	-0.014

* The emulsification assay was performed in the presence of the crude biosurfactant solution (0.1%). After an initial 10-min holding period, absorbance readings were taken every 10 min for 50 min. The log of the absorbance was then plotted versus time and the line slope(decay constant, K_d) calculated.

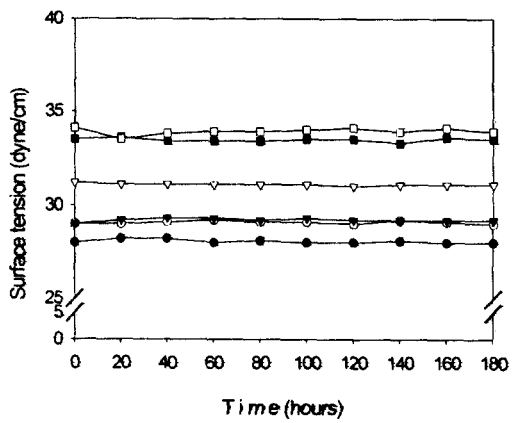

Fig. 3. Effect of temperature on the biosurfactant stability Symbols : -●-, pH 2 ; -○-, pH 4 ; -▼-, pH 6 ; -▽-, pH 7 ; -■-, pH 8 ; -□-, pH 10.

are shown in Fig. 4. The biosurfactant was unaffected by the NaCl and $CaCl_2$ concentrations, although the surface tension did increase slightly with an increased pH. The saline stability of the crude biosurfactant from *Pseudomonas* sp. EL-

G527 suggests that this biosurfactant may be a good candidate for application in marine environments and certain industries related to emulsion.

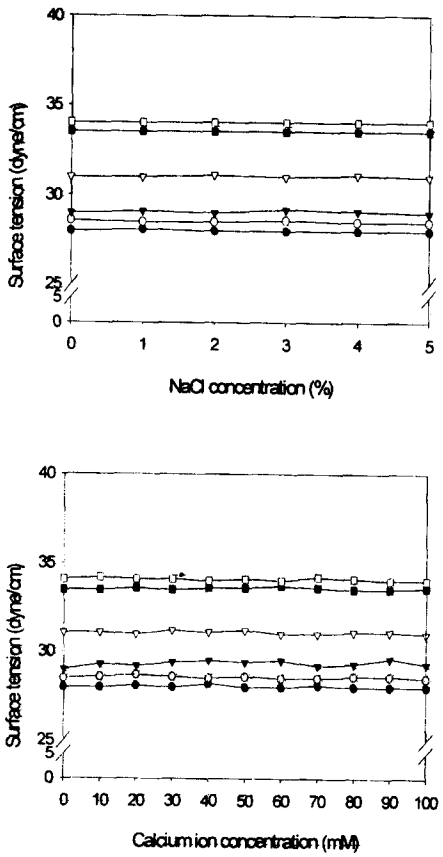

Fig. 4. Effect of Nacl and Calcium ion conentration on the biosurfactant stability. Symbols : -●-, pH 2 ; -○-, pH 4 ; -▼-, pH 6 ; -▽-, pH 7 ; -■-, pH 8 ; -□-, pH 10.

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