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# PRELIMINARY SCREENING OF BIOSURFACTANT-PRODUCING MICROORGANISMS ISOLATED FROM HOT SPRING AND GARAGES IN NORTHERN THAILAND

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## ABSTRACT

Biosurfactants are surface-active compounds produced by microorganisms. These molecules reduce surface tension between aqueous solutions and hydrocarbon mixtures. In this study, we collected soil samples from different hot spring resources in Chiang Mai and Chiang Rai provinces, as well as contaminated soil from garages and screened for biosurfactant-producing microorganisms. Furthermore, we also used stock culturing strains (18 strains of actinomyces and 13 strains of bacteria) from Culture Collection of Excellent Center for Sustainable Development on Bioresources, Chiang Mai University. One hundred and ninety-seven bacterial strains (69 strains from the hot spring and 128 strains from the garage) were isolated and cultured by enriching carbon and nitrogen sources. Each culture medium was sampling to confirm the ability in biosurfactant production. These were conducted using emulsification activity determination (EA), oil spreading technique and parafilm M method. The results reveal that twenty-five strains of bacteria from garage sites presented positive activity which are better than stock bacterial strains. Among these, the emulsifying capacity evaluated by the $E_{24}$ emulsification index range from 7.8-63.3% EA. In addition, the oil displacement area (ODA) was displayed at 9.62-66.50 $cm^2$ and the collapse of droplets on parafilm M method was showed with the average of 5-8 mm. Interestingly, the bacterial isolate (SCMU106) selected from garage site gave the highest values in emulsification activity, oil spreading and parafilm M determination. This will be further investigated in biosurfactant production for health and cosmetics application.

**KEYWORDS:** biosurfactant, surface tension, oil spreading, emulsification index

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# 1. INTRODUCTION

Biosurfactants or microbial surfactants are surface metabolites that produced by bacteria, yeast and fungi having very different chemical structures and properties [1-2]. These biosurfactants are amphiphilic molecules consisting of hydrophobic and hydrophilic domains that find application in an extremely wide variety of industrial process involving emulsification, foaming, detergency, wetting, dispersing or solubilization [3]. Nowadays, biosurfactants are used in industries as a cosmetic and special chemical substances, food, pharmaceutics, agriculture, cleansers, enhanced oil recovery and bioremediation of oil-contaminated sites [4 -5]. They are potential alternatives of chemically synthesized surfactant in a variety of application because of their advantages such as lower toxicity, higher biodegradability, better environmental compatibility, lower critical micelle concentration, each of production, ability to be synthesized from renewable resources, higher foaming, higher selectivity, specific activity at extreme temperature, pH and salinity [2, 6]. In this recent year, the biosurfactants have been placed on the environmental impacts of chemical surfactants and new surfactants for use in any field. The aim of this study is to screen and isolate biosurfactant-producing bacteria from high temperature sites and hydrocarbon contaminated soil at Chiang Mai and Chiang Rai provinces.

# 2. MATERIALS AND METHODS

## 2.1 Isolation and enrichment of biosurfactant-producing microorganisms

Soil samples were collected from Sankamphaeng (Chiang Mai) and Mae Ka Jan (Chiang Rai) hot spring, garages and some strains of bacteria in the experiment were from Culture Collection of Excellent Center for Sustrainable Development on Bioresources, Department of Biology, Faculty of Science, Chiang Mai University. Microorganisms from the soil samples were isolated from liquid enrichment cultures containing 0.1% soy bean oil as a carbon source. One gram of soil sample was incubated into 100 mL of culture medium. The Mckeen medium (20 $gL^{-1}$ glucose, 5.0 $gL^{-1}$ glutamic acid, 1.0 $gL^{-1}$ $K_2HPO_4$, 1.02 $gL^{-1}$ $gMgSO_4$, 0.5 $gL^{-1}$ KCl) supplemented with 1 mL of trace elements solution (0.5 $gL^{-1}$ $MnSO_4.7H_2O$, 0.16 $gL^{-1}$ $CuSO_4.5H_2O$ and 0.015 $gL^{-1}$ $FeSO_4.7H_2O$) adjusting to pH 7.0 was used as cultural medium. The cultures were incubated on rotary shaker (150 rpm) for 3 days at 45 °C (for the hot spring soil samples) and 30°C (for garage sites and culture collection strains). The culture suspension was screened for biosurfactant production by oil spreading test. The bacterial suspension was counted on decimal dilution plate. The biosurtactant – producing bacteria were purified using the Mckeen medium containing soy bean as a carbon and energy source. The isolates were then maintained on nutrient agar [7].

## 2.2 Oil spreading test

The selected strains were compared by measuring of the diameter of the clear zones occured when a drop of a biosurfactant-containg solution is placed on an oil-water surface. The 50 ml of distilled water was added to a large Petri dish (15 cm diameter) followed by the addition of 20 μl of crude oil to the surface of water, 10 μl of supernatant of culture broth. The diameter of clear zones of triplicate assays from the same sample were determined [8].

### 2.3 Emulsification index ($E_{24}$)

The emulsifying capacity was evaluated by an emulsification index ($E_{24}$). The $E_{24}$ of culture samples was determined by adding 2 ml of kerosene and 2 ml of the cell-free broth in test tube, vortexed at high speed for 2 min and allowed to stand for 24h. The $E_{24}$ index is given as percentage of the height of emulsified layer (cm) divided by the total height of the liquid column (cm). The percentage of emulsification index calculated by using the following equation [9-10].

$$E_{24} = \frac{\text{Height of emulsion formed}}{\text{Total height of solution}} \times 100$$

### 2.4 Parafilm M test

The 25 µl of bacterial supernatants when mixed with 1% xylenecyanol were added to the hydrophobic surface of parafilm M. The shape of the drop on the surface was inspected after 1 min. The diameters of droplets were evaluated. The sodium lauryl sulfate and phosphate buffer (pH 7.0) were used as a positive and negative control, respectively [11-12].

## 3. RESULTS AND DISCUSSION

One hundred and ninety-seven bacterial strains were isolated. The diversity of microorganisms was shown in Figure 1. The garage soil sample had higher population of bacteria than hot spring. Twenty-five of one hundred and twenty-eight bacterial strains from garage samples presented oil spreading activity. It was suggested that this site has a variety of hydrocarbon substrates. The bacterial strain SCMU106 showed the highest clear zone and oil displacement area at 66.5 $cm^2$ (Table 1 and Figure 2). While, bacterial isolates SCMU23 and SCMU89 showed lower surfaces activities as lower diameters were found at 40.73 and 38.50 $cm^2$, respectively. This method is better predicted biosurfactant production than the drop collapse method because it is very sensitive for detection [13] and it has several advantages in requiring a small volume of samples. They are rapid and easy to be carried out, and do not require specialized equipment [14]. Thus, the bacterial strain SCMU106 was selected for emulsification index and parafilm M tested.

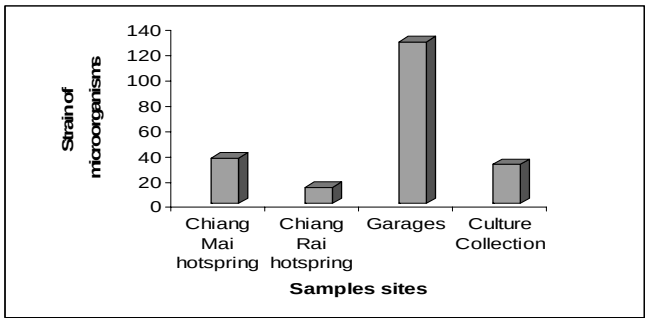


**Figure 1** The diversity of isolated microorganisms for biosurfactant production at different sites

**Table 1** The clear zone of microorganisms on oil surface layer

| Bacterial strains | Clear zone of biosurfactant (cm) | Oil displacement area ($cm^2$) |
|---|---|---|
| SCMU23 | 7.2 | 40.73 |
| SCMU89 | 7.0 | 38.50 |
| SCMU106 | 9.2 | 66.50 |

**Figure 2** The spreading of extracellular-biosurfactant on oil surface layer of bacterial isolate SCMU106

Emulsification activity ($E_{24}$) of the biosurfactant from SCMU106 was measured with kerosene and culture-free broth. $E_{24}$ ranged from 7.8-63.3 EA%. The emulsification of bacterial isolate SCMU106 was detected from the first day of incubation period and showed the highest of emulsion formed at 60 hrs. The degree of emulsification and the stability of the emulsions formed is presented in the Figure 3. The emulsion will expose their stability when it is stored at room temperature. Emulsion layer have been maintaining their form although the duration is more than a week.


**Figure 3** The emulsion form of isolate SCMU106 after various incubation periods (A) emulsion formed at 36 h incubation, (B) emulsion forme at 48 h incubation, (C) emulsion formed at 60 h incubation, (D) 1% Sodium lauryl sulphate (E) Phosphate buffer pH 7

The bacterial isolate SCMU106 showed the highest amounts of extracellular biosurfactant when compared to another isolates (Figure 4B). This methodology proved to be cheap and effective for the screening, maintenance and qualification of biosurfactant-producing bacteria [15]. In addition, It was correlated with surface tension [16].

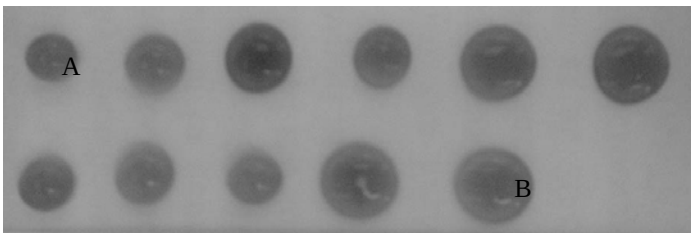


**Figure 4** The activity of surface tension of cell-free broth on parafilm M surface (A)Fresh medium (B) Cell-free broth of bacterial isolate SCMU106

## 4. CONCLUSIONS

The soil sample from garages is a good source for screening of biosurfactant-producing bacteria than hot spring samples. The bacterial isolate SCMU106 displayed the highest activity after detection with oil spreading test, emulsification index and parafilm M method. Identification and optimization for this strain will be further investigated.

## 5. ACKNOWLEDGEMENTS

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