Supplement Information

A lipopetide biosurfactant from Bacilus sp. Lv13 and their combined effects on biodesulfurization of dibenzothiophen

1.1 Screening and culture of strains

A series of concentrations(from 10^{-3} to 10^{-5}) of the bacterial solution were prepared and 0.2 mL of them was spreaded over the surface of the solid medium (added with different concentration of DBT from 1 to 5 mmol / L). And, they were incubated at 50° C for 3 d. Subsequently, some bacterial colonies were chosen to cultivate in 50 mL of liquid medium independently.

1.2 Ion type identification

Ion type identification was carried out with methylene blue-chloroform test. After the supernatants (5 ml) of the broken cell were added into several test tubes, some methylene blue solution (10 mL) and chloroform (5 ml) were added, mixed and standed for a few minutes. If the color (blue) of the upper layer is transferred to the lower layer with the adding of a small amount of 0.2% sodium dodecyl sulfate (SDS), it could be identificated as a nonionic surfactant (Fig.1).

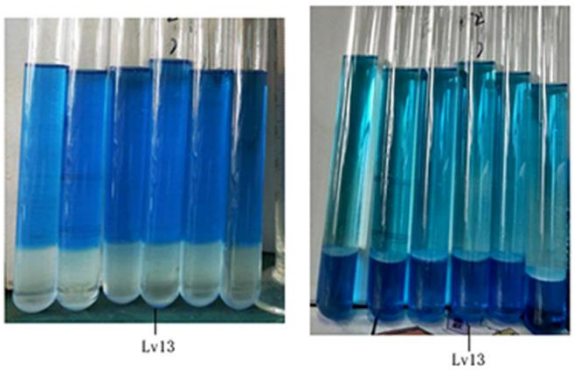

Fig.1 ion type identification (methylene blue-chloroform test)

1.3 TLC determination and refrigeration verification

Thin layer chromatography (TLC) had been applied to identify the biosurfactant. The crude biosurfactant (dissolved in methylene chloride) was spotted onto the silica gel plate with a capillary and put into the saturate tank to chromatograph. And the mixture of chloroform, methanol and water(65:15:2) was used as the developing agents. Chromogenic reagents and its identification component were displayed as follows: phenol-sulfuric acid (glycolipids), ninhydrin (lipopeptides) and bromo thymol blue (phospholipids) (Fig.2). Moreover, the lysate solution was placed in a 4 ° C refrigerator overnight at certain pH value(2-3) to further identify whether it was a lipopeptide. And white flocculent precipitate appeared in its solution at the second day (Fig.3). This further proved that it was a kind of lipopeptides.

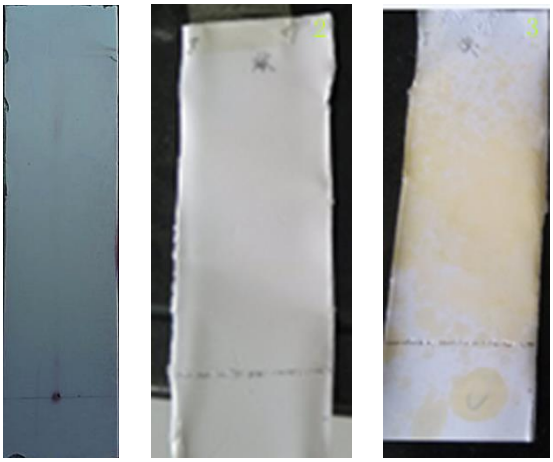

Fig.2 photos of the thin-layer chromatography (TLC) of the biosurfactant

(1) ninhydrin (lipopeptides), (2) phenol-sulfuric acid (glycolipids), (3) bromo thymol blue (phospholipids)

Fig.3 photo of the flocculent precipitate of the biosurfactant after placing in a refrigerator overnight

1.4 Oil-repellent capability

The liquid supernatant or ultrasonic wall-breaking solution was added into the center of the oil film (stained liquid paraffin) respectively to compare the oil-repellent capability (Fig.4).

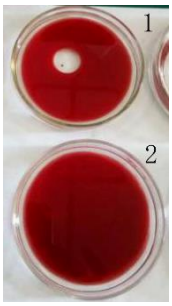

Fig.4 repelled oil capability of biosurfactant solutions (1) ultrasonic wall-breaking solution (2) liquid supernatant

1.5 Component analysis

Component analysis was performed by Agilent 7890/5975C. The flow rate of carrier gas (Helium) was 1.0 mL • min^{-1}. The injection-port temperature was 280° C. And the temperature of column oven was programmed from 60 to 300 ° C at 5° C• min^{-1} which was held for 60 min. GC-MS spectra of the biosurfactant and its speculated compounds were shown as followed (Fig.5). FTIR spectra were measured by using a Nicolet 510P infrared spectrometer to determine the functional groups of the purified surfactant. The solution of the biosurfactant was smeared onto the KBr wafer. And the scanning spectra were 400-4000cm^{-1}. The resolution was 2 cm^{-1}.

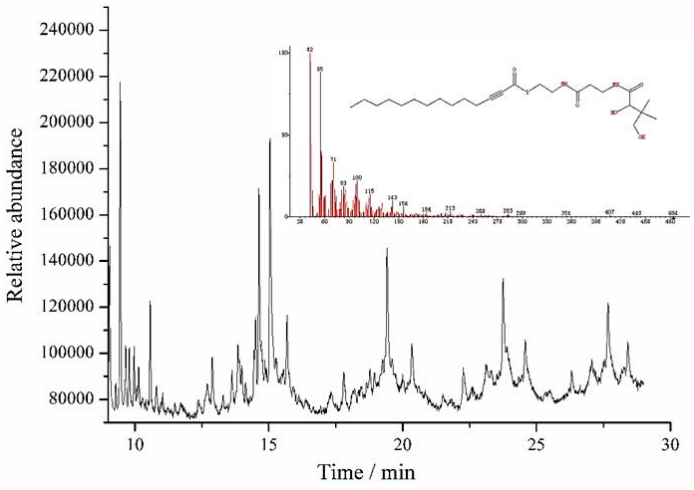

Fig.5 GC-MS spectra of the biosurfactant and its speculated compounds

1.6 CMC value determination

In this experiment, the maximum micelle pressure method was used to determine the critical micelle concentration (CMC) of the extracted biosurfactant. After the leak detection and constant measurement were done, a series of concentrations (0, 0.01, 0.02, 0.03, 0.04 ,0.06, 0.08 g / L) of the crude surfactants solution were chosed to determine differential maximum pressures. And each determination was repeated three times. Finally, the data of concentration and the surface tension were used to analyze the surface tension and CMC value. Finally, the concentration at the turning point of the curve is considered as the CMC value of the biosurfactant (Fig.6).

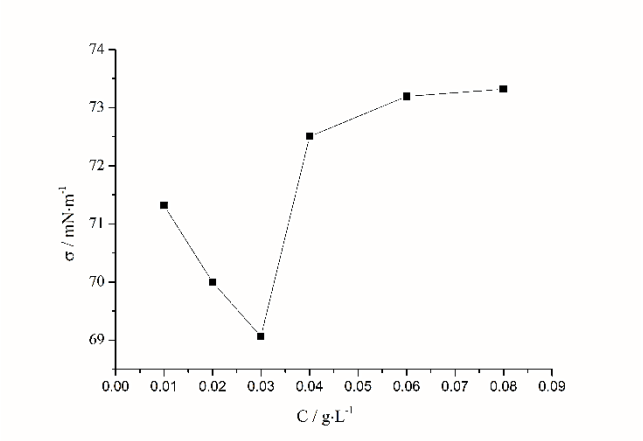

Fig.6 the chart of CMC value determination

1.7 analysis of the ^{1} H NMR spectra

The molecular structure of the surfactant was characterized by NMR instrument (EFT 60) with deuterated chloroform as solvent and TMS as internal standard. But the LP was dissoluted not well and interfered with the impurity, we got a weak NMR Signals (Fig.7). The presence of fatty acids could be indicated by the characteristic shifts in the region of 0.89–1.57ppm. And the presence of N-H could be partial determined by the region of 7.26ppm in spite of the interference of solvent peak.

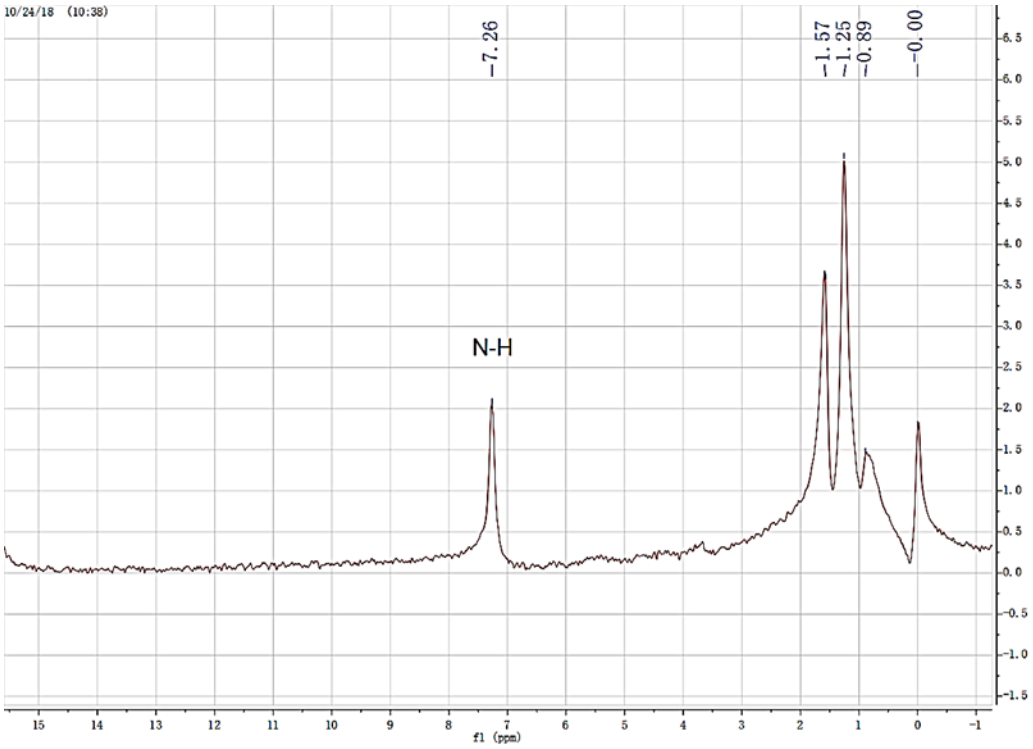

Fig.7 the ^{1}H NMR spectra of LP