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Characterization and Production of Glycolipid Biosurfactant, Mannosylerythritol Lipid from *Candida* sp. SY16

Hee-Sik Kim, Hee-Mock Oh, Yoshiki Tani[1] and Byung-Dae Yoon
Biomolecular Process Engineering Laboratory, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Taejon, Korea
[1]Department of Bioscience, Nara Institute of Science and Technology (NAIST), Nara, Japan

Biosurfactants are microbially produced compounds that exhibit surface activities. Unlike chemical synthetic surfactants, biosurfactants have important advantages, such as biodegradable nature, low toxicity, and various possible structures (Ishigami 1993). With environmental compatibility becoming an increasingly important factor in the selection of industrial chemicals, the use of biosurfactants in environmental applications, such as bioremediation and the dispersion of oil spills, is increasing (Banat *et al.* 2000). In addition, biosurfactants have other uses in the petroleum industry, such as in enhanced oil recovery (Khire and Khan 1994) and transportation of crude oil. However, biosurfactants have not yet been employed extensively in industry because of the relatively high production and recovery costs. To reduce the costs of biosurfactant production, it is necessary to select microorganisms capable of high yield biosurfactant production and to optimize the production and recovery processes (Chayabutra *et al.* 2001).

In this study, a highly productive strain was screened and the chemical structure of biosurfactant that produced by the selected strain was determined by NMR and mass spectroscopic analyses. The culture conditions and some physiological properties for biosurfactants production were also investigated with jar-fermentor. For the efficient production of biosurfactants, the fed-batch cultures were used.

One yeast strain SY16 was selected as a potential producer of biosurfactant for this study by measuring the oil film-collapsing activity. The selected strain was identified as *Candida* sp. SY16. A biosurfactant produced from *Candida* sp. SY16 was purified and confirmed to be a glycolipid. The hydrophilic sugar moiety of the biosurfactant was determined to be -D-mannopyranosyl-(14)-*O-meso*-erythritol by NMR and FAB-MS analyses. The hydrophobic moiety, fatty acid chains, of the biosurfactant was determined to be hexanoic, dodecanoic, tetradecanoic and tetradecenoic acid by GC-MS, and that hexanoic acid occupied the C-2 or C-3 position of the D-mannose unit, and the other position was occupied by tetradecanoic or tetradecenoic acid. Consequently, the structure of the native biosurfactant was determined to be 6-*O*-acetyl-2,3-di-*O*-alkanoyl--D-mannopyranosyl-(14)-*O-meso*-erythritol (MEL-SY16) by NMR analyses (Kim *et al.* 1999).

Candida sp. SY16 reduced the surface tension of the culture broth to 30 dyne/cm and highly emulsified hydrocarbons only when cultured in the vegetable oil-containing media. In the medium containing carbohydrates or hydrocarbons, the production of biosurfactants was hardly observed. In the batch fermentation, MEL-SY16 was produced mainly in the stationary phase of growth, and highly produced in the pH control at 4.0. In the fed-batch culture for MEL-SY16 production, glucose and soybean oil (1:1, w/w) were used in combination as the initial carbon sources for cell growth phase, and soybean oil was used as feeding carbon source at MEL-SY16 production phase. When soybean oil was totally consumed, the vigorous foaming occurred. The soybean oil feeding resulted in the

disappearance of foam and sharply increase of MEL-SY16 production until 200 h, when the concentration of MEL-SY16 was attained to 95 g/l. The foam-stat fed-batch culture for biosurfactants production was first carried out in a laboratory-scale fermentor, where soybean oil was used simultaneously as a carbon substrate and an antifoam agent to extinguish the foam.

MEL-SY16 had the HLB (hydrophilic-lipophilic balance) value of 8.6 and the minimum surface tension of 29 dyne/cm at the CMC (critical micelle concentration) of 15 μM (10 mg/l). In addition, it showed the excellent interfacial tension reducing, emulsifying activity, thermal stability, pH stability, biodegradability, and low toxicity, which suggest the availability of MEL-SY16 in commercial applications.

References

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