Production and Application of Biosurfactant from *Bacillus* MUV4

Onanong Prommachan

Master of Science Thesis in Biotechnology

Prince of Songkla University

2002

Thesis Title Production and Application of Biosurfactant from *Bacillus* MUV4

Author Miss Onanong Prommachan

Major Program Biotechnology

Academic Year 2002

Abstract

Factors affecting the production of biosurfactant by *Bacillus* MUV4 cultivated in Mckeen medium (pH 7.0) at 30°C were investigated. The maximum biosurfactant was obtained after 48 h cultivation with cell growth, oil displacement area (ODA) and emulsification capacity (EC) values was 5.78 (OD_{660}), 9.76 cm^2 and 0.87%, respectively. Two point five percent of glucose was the best carbon source for biosurfactant production. Among the nitrogen source (0.5%) tested, L-glutamic acid and monosodium glutamate gave higher oil ODA and EC values but no significant difference in ODA and EC values were detected among L-glutamic acid and monosodium glutamate treatment. Therefore, monosodium glutamate with a concentration of 1.0% was selected for further study. Addition of 0.3% yeast extract in the medium improved growth and higher ODA, emulsification activity (EA) and EC values. Cultivation the organism in the optimal medium contained 2.5% glucose, 1.0% monosodium glutamate and 0.3% yeast extract improved the growth and biosurfactant production with higher ODA, EA and EC values (78.5 cm^2, 81.82% and 5.18%, respectively). Comparison on growth and biosurfactant production in the basal medium and optimized media with initial pH 7.0 at 30°C revealed that the growth was increased 1.9 folds and the ODA and EC increased 8.0 and 5.8 folds, respectively. Uncontrolled pH during cultivation in a fermentor gave higher ODA, EA and EC than those under controlled pH starting at 7.0 condition. An increase in the aeration rate from 0 to 1.0 vvm at agitation

speed of 200 rpm could elevate biosurfactant production. The partial purification of biosurfactant was performed by precipitation of 60 h culture supernate with 6 N HCl, neutralized with 2.0 N NaOH to pH 7.0 and freeze-drying. The acid precipitated biosurfactant yield was 0.8 g/l. This acid precipitated biosurfactant was soluble in water, alkaline water, methanol, ethanol, ethyl acetate, acetonitrile, acetone and chloroform but was insoluble in hexane. pH had much effect on ODA and EC. Relative ODA and EC of culture broth was stable at the pH range 6.0-10.0 while relative EA was stable at the pH range 4.0-14.0. Relative ODA, EA and EC of acid precipitated biosurfactant were stable at the pH range 6.0-12.0. The maximum ODA, EA and EC values retained more than 80% at pH 8.0. NaCl concentration had much effect on ODA and EA. At 15-20% NaCl the relative ODA was less than 10% and EA was not detectable while the relative EC was higher than 60% in culture broth and 25% in acid precipitated biosurfactant. Temperature had much effect on ODA and EC than EA. Even at 100 °C for 12 h the relative EA of the biosurfactant in culture broth still retained activity more than 80%.

The biosurfactant from *Bacillus* MUV4 was preliminarity characterized by TLC analysis and chemical tests. The compound included lipid and ninhydrin-positive compounds. The biosurfactant showed antimicrobial activity against the growth of *Bacillus anthracis, B. subtilis, Shigella* sp. and *Streptococus faecalis* ATCC 29212 but not against the growth of *E. coli, P. aeruginosa, Salmonella* sp. and *Staphylococcus aureus*. Effect of the acid precipitated biosurfactant (0.1%) enhanced on recovery of kerosene oil from sandpack column was 50.04%.