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Production of Biosurfactant by *Pseudomonas aeruginosa* EMS1 from Soybean Oil and Whey

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Pseudomonas aeruginosa EMS1, isolated from activated sludge, was able to grow and produce a biosurfactant on 4.5% soybean oil, used as the source of energy and carbon. *Pseudomonas aeruginosa* EMS1 was cultivated at 30℃ in a reciprocal shaking incubator, and the highest biosurfactant production was observed after 3 days. Furthermore, *Pseudomonas aeruginosa* EMS1 was also able to use whey as a co-substrate for biosurfactant production and growth

Key words : biosurfactant, emulsifying activity, *Pseudomonas aeruginosa* EMS1, soybean oil, whey

1. Introduction

The industrial need for surfactants is constantly growing. Surfactants possess both hydrophilic and hydrophobic structural moieties, which in turn impart many unusual properties, including an ability to lower the surface tension of water[1]. The commercial importance of surfactants can be evidenced from their increased production and number of industrial applications. Biosurfactants have attracted recent attention as promising natural surfactants because they offer several advantages over chemical surfactants, such as lower toxicity, biodegradability, and ecological acceptability[2,3]. Interest in biosurfactants is increasing because they have potential for several types of commercial applications.

At present, the economics of biosurfactant production have not received much attention, however, economic strategies must be devised if biosurfactants are to compete with chemical surfactants. Choosing inexpensive raw materials is key to the overall economics of the process because raw materials account for almost fifty percent of the final product cost of a biosurfactant. The food industry produces a considerable amount of by-products that are still rich in organic substances, for example, cheese whey and cottage cheese whey, which are the major by-products of the cheese-making industry. Cheese whey is still rich in proteins, lactose, vitamins, and minerals[4], and is already used in a variety of valorization processes, including drying and constituent extraction[5] in the production of biomass[6~8], butanol[9], and ethanol[10]. However, owing to high collection costs and seasonal fluctuations in production, cheese whey is more often discarded in sewage or in the environment, thereby causing water and soil pollution[11,12].

This paper describes the growth characteristics and biosurfactant production of the newly isolated *Pseudomonas aeruginosa* EMS1 on various carbon sources, plus the effect of whey supplementation.

2. Materials and Methods

2.1 Microorganism and cultivation conditions

The microorganism used in this study was *Pseudomonas aeruginosa* EMS1, which was isolated from activated sludge. A nutrient broth was used for the preparation of the inoculum. The cultures were grown in this broth for 18h at 30℃ (OD_{660nm} 1.0). The inoculum was used at a 2%(v/v)

level. The optimum mineral salts medium used for the growth of the biosurfactant-producing bacterium was as follows: NH_4NO_3 3g/l, K_2HPO_4 0.3g/l, KH_2PO_4 0.3g/l, $MgSO_4 \cdot 7H_2O$ 0.2g/l, and $CaCl_2 \cdot 2H_2O$ 0.25g/l. The pH of the medium was adjusted to 7.0, then the medium was sterilized by an autoclave. The *Pseudomonas aeruginosa* EMS1 was grown for 5 days in 50 ml of a mineral salts medium containing 2% (w/v) soybean oil as the sole source of carbon at 30℃ in a 250-ml Erlenmeyer flask.

To investigate the growth characteristics and biosurfactant production on different carbon sources, carbohydrates, hydrocarbons, and vegetable oil were used as substitutes for soybean oil in the mineral salts medium.

2.2. Maintenance and identification of bacterium

The microorganisms were stored at -70℃ in glycerol and subcultured on tryptic soy agar plates before use as an inoculum. The selected bacterial isolate was characterized using API 20E strips (bioMerieux Vitek, Inc., Hazelwood, Mo), several physiological and biological tests, and a 16S rDNA analysis[13].

2.3. Biomass determination

The biomass concentration was determined on the basis of the dry cell weight. Five milliliters of culture broth were mixed with 10 ml of methanol/chloroform(1:2). The mixture was shaken and centrifuged at 12000×g for 20 min to form a biphasic solution. The upper phase was discarded, then the lower phase(solid) was washed with 0.85% NaCl, placed in a dry oven at 105℃ for 48h to dry the cells, and weighed.

2.4. Analytical Methods

The surface tensions of the culture supernatant and crude biosurfactant were determined using a Tensiometer(Fisher Scientific, Surface Tensiotat 21). The biosurfactant concentration was estimated by determining the factor necessary for the culture supernatant to reach the critical micelle concentration(CMC)[14]. If the concentration of the biosurfactant falls bellow the CMC, the surface activity will depend on the concentration of the surface active molecules, as such, the value of the surface tension will increase. Accordingly, this dilution factor(Fcmc) is a direct measure of the biosurfactant concentration. To measure the emulsifying activity, the samples being tested(0.5ml) were introduced into a 50ml Erlenmeyer flask containing distilled water to a final volume of 7.5ml, then 1ml of soybean oil was added. The samples were incubated with shaking at 30℃ for 1h. The turbidity was determined at 540nm[15].

3. Results and Discussion

3.1. Screening for biosurfactant-producing microorganisms

When grown in a mineral salts medium with soybean oil as the sole substrate, ten strains of biosurfactant-producing microorganisms decreased the culture medium surface tension to below 35mN/m and also stabilized the oil-water emersions. The microorganism that displayed the highest biosurfactant productivity was then selected for a more detailed analysis. As a result, it was identified as *Pseudomonas aeruginosa* using API 20E strips, several biological and physiological tests, and a 16S rDNA analysis.

3.2. Effect of soybean oil concentration on biosurfactant production

Pseudomonas aeruginosa EMS1 was grown on different concentrations of soybean oil. The biosurfactant production was measured based on the emulsifying activity and Fcmc. As shown in Figure 1, a 4.5%(w/v) concentration of soybean oil was found to be the optimal level for biosurfactant production. The emulsifying activity and Fcmc increased almost linearly with an increasing soybean oil concentration up to about 5%(w/v). Thereafter, a further increase in the soybean oil concentration(up to 8%) caused a slight decrease in the emulsifying activity.

3.3. Time course of biosurfactant production and cell growth

Figure 2 shows the pattern of biosurfactant formation and cell growth of *Pseudomonas aeruginos* EMS1 in a medium containing 4.5%

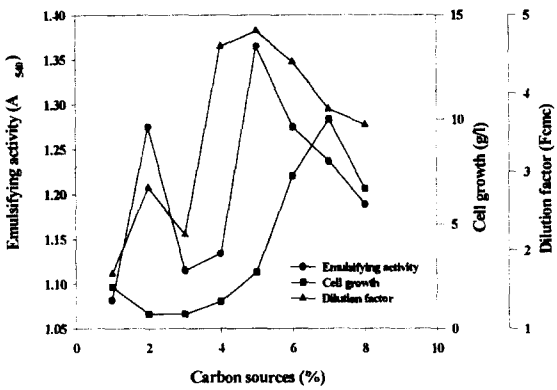

Fig. 1. Effect of soybean oil concentration on biosurfactant production.

soybean oil. After 40 hours, *Pseudomonas aeruginosa* EMS1 started to produce a biosurfactant along with its cell growth. The maximum biosurfactant production occurred during the late exponential phase of the culture. *Pseudomonas aeruginosa* GS3 has been reported to produce a biosurfactant under growth-limiting conditions when its cells reach the stationary phase[2]. However, the growth-associated production of emersifiers has been reported for several other microorganisms[14~16].

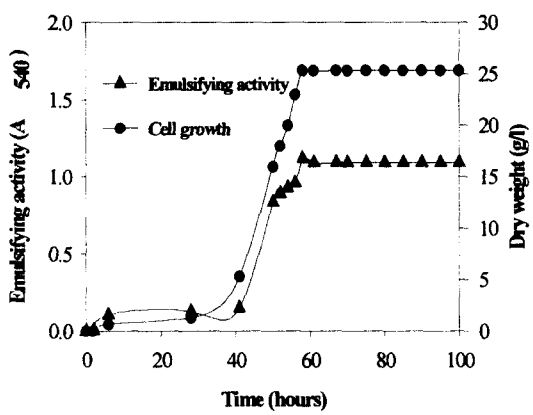

Fig. 2. Time course of cell growth and biosurfactant production by *Pseudomonas aeruginosa* EMS1.

3.4. Effect of whey supplementation

An inexpensive potential source for substrates is waste streams. Plus, waste treatment costs can be offset by the production of valuable co-products. Such sources of carbon can often be obtained at little or no cost. Currently, there is a great deal of interest in using industrial wastes as nutrient sources in bioconversions. Accordingly, this study screened the whey produced during cheese processing for its feasibility as a co-substrate for biosurfactant production. Just 4.5% soybean oil and whey were added to distilled water, then the cell growth and biosurfactant production were examined. The results, presented in Figure 3, confirm that *Pseudomonas aeruginosa* EMS1 utilized the whey as a co-substrate for biosurfactant production and growth, as the biosurfactant produced from the single substrate, including its quality, was unchanged. The highest cell growth and emulsifying activity were obtained when 2% whey was added to 4.5% soybean oil basal media. Accordingly, these data demonstrate the potential for using whey and soybean oil for the commercial fermentation production of biosurfactants.

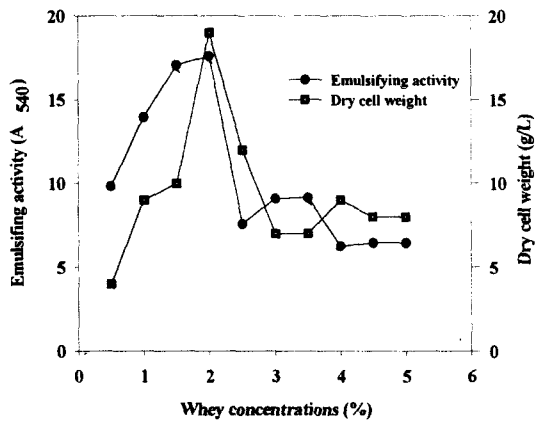

Fig. 3. Effect of whey supplementation on production of biosurfactant by *Pseudomonas aeruginosa* EMS1.

3.5. Growth characteristics and biosurfactant production on different carbon sources

The source of carbon used in bacterial cultures is very important in biosurfactant production. Generally, the carbon sources used in biosurfactant production can be divided into three categories: carbohydrates, hydrocarbons, and vegetable oils. Some microorganisms can produce biosurfactants using hydrophobic carbon sources, hydrocarbons,

or vegetable oils, while others only use carbohydrates or a combination of several carbon sources. In this study, several low-cost substrates were used to produce a biosurfactant so as to reduce the final product cost. As shown in Table 1, the highest yield was obtained from vegetable oil. *Pseudomonas aeruginosa* EMS1 was able to grow or produce a biosurfactant when the hydrocarbons *n*-octadecane and *n*-hexadecane were supplied as the carbon source.

Table 1. Effect of carbon source on biosurfactant production

Carbon source(2%)	Growth (g/L)	Emulsifying activity (540nm)	Dilution facter (Fcmc)
fructose	10.0	0.321	1.0
n-hexadecane	8.3	0.132	1.4
n-octadecane	10.0	0.350	1.0
toluene	1.7	0.111	1.0
crude oil	1.7	0.104	1.0
bunker A	3.0	0.065	1.0
bunker B	3.0	0.122	1.0
bunker C	6.7	0.403	1.0
olive oil	13.0	0.498	1.0
soybean oil	28.0	1.165	3.7
corn oil	28.0	0.878	1.0
peanut oil	25.0	1.182	3.4
oleic acid	5.0	0.969	1.8

4. Conclusion

Pseudomonas aeruginosa EMS1 is a very efficient biosurfactant producer, and its culture conditions are relatively inexpensive and economical. The use of cheap substrates, development of less expensive processes, and achievement of high biosurfactant yields are the major factors that govern the successful utilization of biosurfactants on a large scale. Accordingly, the results from the current preliminary study show this to be possible.

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References

[1] Desai JD, Banat IM(1997) Microbial production of surfactants and their commercial potential. Microbiol. *Mol. Biol. Rev.* **61** : 47 ~64.

[2] Patel RM, Desai AJ (1997) Biosurfactant production by *Pseudomonas aeruginosa* GS3 from molasses. *Lett. Appl. Microbiol.* **25** : 91~94.

[3] Torabizadeh H, Shojaosadati SA, Tehrani HA (1996) Preparation and characterization of bioemulsifier from *Saccharomyces cerevisiae* and its application in food products. *Food Sci. Technol.* **29** : 734~737.

[4] Moulin G, Galzy P (1984) Whey, a potential substrate for biotechnology. *Biotechnol. Genet. Eng. Rev.* **1** : 347~373.

[5] Rosner G (1982) Operation Rhonc-Alpes de valorization des sous-produits de l'agroalimentairc par l'alimentation animalc, 2emc rapport, Etablissement Publiquc Rcgional Rhonc-Alpcs, Lyons, France.

[6] Champagne GP, Goulet J, Lachance RA (1990) Production of baker's yeast in cheese whey ultrafilterate. *Appl. Environ. Microbiol.* **56** : 425~430.

[7] Michel A, Jacob F, Perrier J, Poncet S(1987) Yeast production from crude sweet whey. *Biotechnol. Bioeng.* **30** : 780~783.

[8] Tahoun MK, El-Merheb Z, Salam A, Youssef A(1987) Biomass and lipids from lactose or whey by *Trichosporon beigelii*. *Biotechnol. Bioeng.* **29** : 358~360.

[9] Maddox IS (1980) Production of n-butanol from whey filtrate using *Clostridium acetobutylicum* N.C.I.B. 2951. *Biotechnol. Lett.* **2** : 493~498.

[10] Porro D, Martigani E, Ranzi BM, Alberghina L(1992) Lactose/whey utilization and ethanol production by transformed Saccharomyces cerevisiae cells. *Biotechnol. Bioeng.* **39** : 799 ~805.

[11] Ghaly AE, Singh RK(1989) Pollution potential reduction of cheese whey through yeast

fermentation. *Appl. Biochem. Biotechnol.* **22** : 181~203.

[12] Glazer AN, Nikaido H(1995) Disposable of whey. A case study, p. 44~46. in A. N. Glaze and H Nikaido(ed.), Microbiol biotechnology. Fundamentals of applied microbiology. W. H. Freeman and Company, New York, N. Y.

[13] Krieg NR, Holt JG(ed.) (1984) Bergey's manual of systematic bacteriology, vol. 1. Williams & Wilkins, Baltimore.

[14] Duvnjak Z, Kosaric N(1985) Production and release of surfactant by *Corynebacterium lepus* in hydrocarbon and glucose media. *Biotechnol. Lett.* **7** : 793~796.

[15] Johnson V, Singh M, Saini VS, Adhikari DK, Sista V, Yadav NK(1992) Bioemulsifier production by an oleaginous yeast *Rhodotorula glutinis* IIP-30. *Biotechnol. Lett.* **14** : 487~490.

[16] Kim H-S, Lee C-H, Suh H-H, Ahn K-H, Oh H-M, Kwon G-S, Yang J-W, Yoon B-D (1997) A lipopeptide biosurfactant produced by *Bacillus subtillis* C9 selected through the oil film-collapsing assay. *J. Microbiol. Biotechnol.* **7** : 180~187.