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Lincomycin Production in the culture of *Streptomyces lincolnensis* using crude soybean oil in air lift bioreactor

Ki-An Cho[1] · Hoon Cho[2*]

[1]*Department of Environmental health, Cho-dang University, 419, Muan-kun, Chonnam 534-800, Korea.*

[2]*Department of Polymer Science & Engineering, Chosun University, Gwangju 501-759, Korea*

Abstract

Using crude soybean oil as the sole carbon source, the lincomycin production from Streptomyces lincolnensis LC 345 was investigated in the air lift bioreactor. When 30 g/L of crude soybean oil was used, the maximum lincomycin concentration reached 0.89 g/L, after 5 days of culture. When CSL concentration was increased from 10 to 30 g/L, Lincomycin concentration was increased from 0.6 to 1.2. On the other hand, when CSL concentration was increased from 40 to 60 g/L, it was decreased from 1.15 to 0.7 g/L. Using these results, fed batch cultures for comparing the use of crude soybean oil and glucose as a conventional carbon source were carried out in a 5 L air lift bioreactor. When crude soybean oil was used as the sole carbon source, the maximum lincomycin concentration was 2.0 g/L, which was about 2.0 fold higher than that of glucose medium after 7 day of culture. The product yield from olive oil was 0.042 g/g consumed carbon source, which was about 3.8 fold higher than that of glucose.

Key words : Crude soybean oil, Lincomycin, *Streptomyces lincolnensis*

* Corresponding author E-mail : hcho@chosun.ac.kr

Ⅰ. Introduction

Vegetable oils such as olive, cottonseed oil, soybean oil, rapeseed oil, peanut oil, and corn oil contained approximately 2-4 folds the energy source of glucose or sucrose on a per weight basis. In addition, they contained 2.0 fold the amount of carbon when compared to glucose on a weight basis and are somewhat cheaper than others sugars such as glucose and sucrose on per weight of active ingredient basis. At a volume required to add 10^4 kcal energy, they require only about 25% of the volume of sugars or molasses and 5% of thevolume of a starch addition[1]. Oils are often cheap carbon sources compared to carbohydrates such as glucose and sucrose and are an excellent product extractor. Eiki *et al*reported that since josamycin was distributed in the oily phase of the culture broth, it was removed from the culture broth, and they succeeded five fold higher than without using an oily carbon source in producing josamycin concentrations[2]. Pan *et al*also reported that a complex medium containing methyloleate as the primary carbon source was developed for commercial production of cephalosporin by *Cephalosporium acremonium* BC-2116[3]. Furthermore, various lipids including vegetable oils, long chain fatty acid, and animal oil as the carbon source for an efficient kasugamycin production by *Streptomyces kadugaensis* were investigated[4]. Paul *et al* reported a 54% higher cephalosporin C production when 20g/l of sesame oil was added to the culture broth using *Cephalosporium acremonium*[5]. Recently, we found that *Streptomyces lincolnensis* LC 345 consumed vegetable oils and produced a large amount of lincomycin. We are attempting to confirm the possibility of enhancing the production of compactin using an air-lift bioreactor, because air-lift bioreactors have many economical implications in regard to reactor construction, maintenance and scale-up as previously reported[6,7]. However, the ability of air-lift bioreactors to supply oxygen is generally lower than that of the conventional type of bioreactors, *i.e.*, aeration and stirred tank bioreactor. Therefore, it is necessary to determine the optimal culture conditions that makes suitable for an air-lift bioreactor to operator.

In this study, for efficient production of lincomycin from *Streptomyces lincolnensis* LC 345,various concentrations of crude soybean oil and CSL concentration were investigated in flasks. After obtaining these results, fed batch cultures for comparing the use of crude soybean oil and glucose as the conventional carbon source were carried out in air lift bioreactor.

II. MATERIAL AND METHODS

Microorganism, media, and culture conditions

The strain used in this study was *Streptomyces lincolnensis* LC 345. The composition of the second seed medium was as follows (g/L): olive oil, 5; starch, 5: yeast extract, 5; soybean meal, 5; $NaNO_3$, 0.5; $MgSO_4 \cdot 7H_2O$, 0.5. For production of lincomycin in a flask and air lift bioreactor, the following medium was

used (g/L); crude soybean oil, 30; CSL, 15; soybean meal, 15; KOH, 0.5; K_2HPO_4, 0.25; $MgSO_4 \cdot 7H_2O$, 0.5; trace elements solution, 3ml. Trace elements solution contained following ingredients (ppm): $FeCl_3$, 500; $ZnCl_2$, 600; $MnCl_2$, 1,00; $CoCl_2$, 300. All the media components were sterilized at 121℃nd 1.2 atm for 20 min. The pH of the media was adjusted to 6.9 before sterilization. One looful of *Streptomyces lincolnensis* LC 345 was transferred to the slant medium and cultured at 28℃or 7 days. Then, one looful of the slant culture of *Streptomyces lincolnensis* LC 345 was inoculated into a 500ml Erlenmeyer flask containing 50ml of the first seed medium and cultured at 28℃ for 1 day on a reciprocating shaker at 120 rpm. For the second seed, 2.5 % of the first seed was inoculated into a 500ml Erlenmeyer flask containing 50ml of the second seed medium and cultured at 2 8℃for 1 day on a reciprocating shaker at 120 rpm. For the production of lincomycin, 5 % of the second seed was inoculated into a 500ml Erlenmeyer flask containing 50ml of the production medium or into a 5 air lift bioreactor containing 2L of production medium, and cultured at 28℃.

Cell and oil concentration

The cell concentration was determined by the intracellular nucleic acid (INA) concentration. The oil concentration was measured by a solvent extraction method [6]. Three milliliters of culture broth were mixed with 6ml of n-hexane and the mixture was vigorously shaken for 2 min in a capped Erlenmeyer flask and then centrifuged at 3,000 rpm for 15min. The upper hexane layer was removed and dried at 80℃for 3hr, and the residue was weighed to determine the extracted oil weight.

Glucose concentration

Glucose concentration as the total sugar was measured by the phenol-sulfuric acid method reported by Dubois *et al*[8].

Lincomycin concentration

The lincomycin concentration was measured by the microbial assay, using *Sareina lutea* ATCC 9341 as a test organism.

III. RESULT AND DISCUSSION

Lincomycin, an antibiotic produced by *Streptomyces lincolnensis var lincolnensis*, has been used as a feed supplement to promote the growth of animals and birds, either alone or in combination with antibiotics. It has also been used as an industrial preservative, for example, as a bacteria static rinse for laundered clothes, and for impregnating paper and fabrics. It has also been useful for suppressing the growth of sensitive organisms in plates assays, and other biological media. Oils were first used as carriers for antifoams in antibiotic production processes because the surface tension at the liquid-gas interface in a liquid medium is low. Moreover, the concentrations of these

oils in a culture broth are maintained at a low level since they are hydrophobic[9]. Choi *et al* previously investigated various vegetable oils and animal oils as the sole carbon source for efficient tylosin production in the culture of *Streptomyces fradiae* T 1555. There was a 1.6 or 7.0-fold increase in tyrosineproduction when rapeseed oils were used compared with using starch and glucose at the same initial concentration[10]. In order to increase the oxygen transfer coefficient in the culture of tetracycline-producing *Streptomyces aureofaciens* using an air-lift bioreactor, the various vegetable oils and hydrocarbons were used. In the case of soybean oil or dodecan addition, the oxygen transfer coefficient was highest[11]. Recently, we found that *Streptomyces lincolnensis* LC 345 consumedvegetable oils and produced a large amount of lincomycin.

In order to investigate the effects of various crude vegetable oils on the production of lincomycinproduction, rapeseed oil, soybean oil, olive oil, palm oil, corn oil, sunflower oil, and cottonseed oil were used. Batch cultures were carried out in flasks containing 50ml of the production basal medium, with 20 g/l of each crude vegetable oil for 5 days. The production of lincomycinare shown in Table 1.

Table 1. Effect of various crude vegetable oils on Lincomycin concentration.

Crude Vegetable oils	Lincomycin concentration (Relative, %)
Rapeseed oil	92.2
Soybean oil	99.2
Olive oil	100
Palm oil	22.0
Corn oil	88.2
Sunflower oil	90.1
Cottonseed oil,	88.2

Among the various crude vegetable oils, when olive oil was used, the lincomycin production was higher than other vegetable oils. However, soybean oil was the best carbon source for producing the lincomycin because it was very cheap compared to other vegetable oils. In order to determine the optimal initial soybean oil concentration for the effective production of lincomycin, initial oil concentrations of 10, 20, 30, 50, 60 and 70 g/L were used in flask cultures for 5 days. Theoil consumption and lincomycin production are shown in Table 2.

Table 2. Effect of crude soybean oil concentrate on Lincomycin concentration and oil consumption.

Crude soybean oil concentration (g/L)	Lincomycin concentration (g/L)	Residual oil concentration (g/L)
10	0.35	0
20	0.66	0
30	0.89	5.2
40	0.75	9.9
50	0.70	21.9
60	0.62	35.6
70	0.50	51.2

The cell concentrations were similar when either 20 or 30 g/L of soybean oil was used. However, with above 50 g/L of soybean oil, the cell concentrations decreased with increasing oil concentrations (data not shown). An initial concentration of 30 g/L gave the highest lincomycin concentration at 0.89 g/L. The concentration of soybean oil consumed was 24.8 g/L. When below 20 g/L of soybean oil was used, the oil was entirely consumed. However, when 40, 50, 60 or 70 g/L of oil was used, the concentrations of oil consumed were 30.1, 28.1, 24.4 and 18.8 g/L, respectively, with lincomycin

concentrations of 0.75, 0.70, 0.62 and 0.50 g/L, respectively. When the initial soybean oil concentration was 30 g/L, the maximum product yield was 0.036 g/g of oil consumed.

Nitrogen sources have long been known to suppress the biosynthesis of a variety of chemically unrelated antibiotics and other secondary metabolites. The most common observation is a decrease in the level of antibiotic produced in the presence of an excess of the nitrogen source. For effective antibiotic production, many authors have reported that the type and concentration of various nitrogen sources in the growth medium have been affected[12,13]. Previously, nitrogen sources, such as pharmamedia and gluten meal, were applied in our laboratory for the effective production of tylosin from *Streptomyces fradiae*, as these nitrogen sources contain various amino acids. In the case of compactin production, soybean meal and pharmamedia, in an air-lift bioreactor culture using *Penicillium citrinum* L-18065, were found to be the most suitable nitrogen sources. They are also cheap and commercially available sources for fermentation processes[14,15]. CSL as the sole nitrogen source were very cheap. Therefore we investigated the concentrationsof CSL fro effective lincomycin production in flask culture. The results are shown in Table 3.

Table 3. Effect of CSL concentration on lincomycin production.

CSL concentration (g./l)	Lincomycin concentration (g/l)
10	0.6
20	0.95
30	1.2
40	1.15
50	0.8
60	0.7

When CSL concentration was increased from 10 to 30 g/L, Lincomycin concentration was increased from 0.6 to 1.2. On the other hand, when CSL concentration was increased from 40 to 60 g/L, it was decreased from 1.15 to 0.7g/L.

In order to compare the use of crude soybean oil and glucose on the lincomycin production, fed batch cultures carried out in 5L air lift bioreactor containing 2 L of production medium for 7 days. The cell growth, carbon source concentration, and lincomycin production are shown in Figure 1.

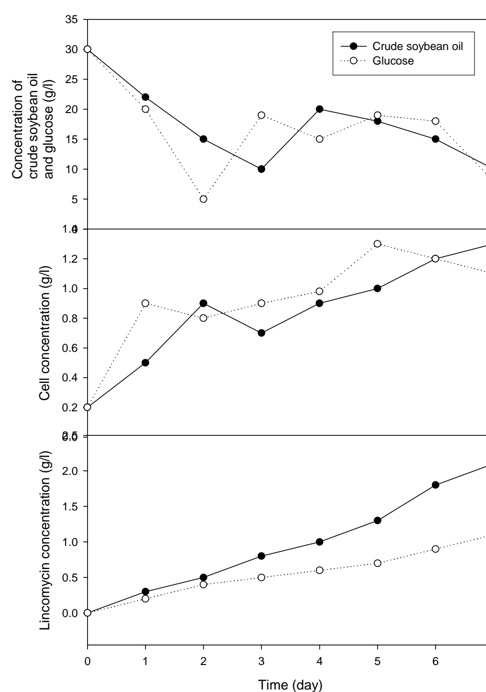

Fig. 1. Comparison of crude soybean oil and glucose on the lincomycin production, cell growth, and carbon source concentration.

The feeding of oil and glucosewas carried out after 2 and 4 days of culture, respectively. With CSL, the feeding was carried out after 3 days of

culture. When crude soybean oil was used, 50.2 g/Lof olive oil were consumed after 7 days of culture. The maximum lincomycin concentration was 2.1 g/L after 7 days of culture. The cell concentration ranged from 0.8 to 1.5 after 2 days of culture. On the other hand, In the case of glucose medium, 88.3 g/L of glucose were consumed, but the produced maximum lincomycin concentration was 1.0 g/L after 7 days of culture. The product yield from olive oil and glucose were 0.042 and 0.011g/g consumed carbon source, respectively. In some cases of industrial antibiotic production, starch or glucose has been used as a carbon source. However, in the case of lincomycin production by *Streptomyces lincolnensis*, the yield using crude soybean oil was markedly higher than that of glucose. This indicates that crude oil is the most suitable carbon source for an efficient lincomycin production from *Streptomyces lincolnensis*.

IV. CONCLUSION

This research is to investigate the feasibility on lincomycin production in the culture of *Streptomyces lincolnensis* using crude soybean oil as the sole carbon source and CSL as the sole nitrogen source in the air lift bioreactor. Various vegetable oils, and nitrogen source, crude soybean oil and CSL were the most suitable energy one. We are now screening mutants that are resistant to high concentrations of unsaturated fatty acids. Moreover, we are investigating the fish oils as the sole carbon source instead of vegetable oils. There are plenty of fish oils that are very cheap and more difficult to dispose of them. If fish oils can be used as the carbon source, the product costs of lincomycin will be cut.

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