J. Microbiol. Biotechnol. (2004), **14**(3), 532–539

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Effect of Carbon Source Consumption Rate on Lincomycin Production from *Streptomyces lincolnensis*

CHOI, DUBOK AND KIAN CHO*

Department of Environmental Engineering, Cho-dang University, Chonnam 534-800, Korea

Received: August 18, 2003
Accepted: February 7, 2004

Abstract For efficient lincomycin production from *Streptomyces lincolnensis* L1245, various vegetable oils, natural nitrogen sources, and surfactants were investigated at the pilot-scale level in the flask. Olive oil as the sole carbon source was the most suitable one for producing lincomycin. When 20 g/l of olive oil was used, the lincomycin concentration and lipase activity reached 1.01 g/l and 182 U/ml, respectively, after 5 days of culture. Among the various unsaturated fatty acids, when linolenic acid was used, the cell growth and lincomycin production were markedly decreased. On the other hand, when 0.2 g/l of oleic acid was added to the culture broth, the maximum lincomycin concentration was 1.0 g/l, which was about 1.7-fold higher than that obtained without the addition of oleic acid. Among the various natural nitrogen sources, pharmamedia or soybean meal was the most suitable nitrogen source. In particular, in the case of a mixture of 10 g/l of pharmamedia and soybean meal, 1.5 g/l of lincomycin concentration and 220 U/ml of lipase activity were obtained. When Span 180 was used as the surfactant, lincomycin production, lipase activity, and oil consumption increased. The correlation between the consumption rates of oil and lincomycin production in a culture using olive oil as the sole carbon source was also investigated. The lincomycin production depended on the consumption rate of olive oil. Using these results, fed-batch cultures for comparing the use of olive oil and starch as a conventional carbon source were carried out in a 5-l fermentor. When olive oil was used as the sole carbon source, 34 g/l of olive oil was consumed after 7 days of culture. The maximum lincomycin concentration was 3.0 g/l, which was about 2.0-fold higher than that of starch medium after 7 days of culture. The product yield was 0.09 g/g of consumed carbon source, which was about 3.0-fold higher than that of starch medium after 7 days of culture.

Key words: Olive oil, lincomycin, *Streptomyces lincolnensis*

*Corresponding author
Phone: 82-61-450-1264; Fax: 82-61-450-1979;
E-mail: Kacho@chodang.ac.kr

The biosynthesis of most antibiotics is strongly regulated by the nature and the concentration of carbon, nitrogen, phosphorus, and trace elements [11, 22, 24, 28]. It has been reported that high concentrations of an easily metabolized nutrient would reduce antibiotic titer and postpone its onset through catabolite repression [8]. Rapid catabolism of carbon has caused a decrease in the rate of biosynthesis of many antibiotics [10]. Industrial antibiotic production has therefore been carried out using a carbon source such as lactose, maltose, or galactose, which is slowly utilized, or by slowly adding glucose to the fermentation broth to obtain a slow growth rate for penicillin biosynthesis [26]. Similarly, the continuous feeding of glucose to the fermentation broth stimulated the production of the polyene macrolide antibiotics such as candidin and candihexin [19]. However, there are technical and economic problems because of the complexity and contamination involved in these fermentation processes.

Nowadays, in order to solve these technical and economic problems, various vegetables or fish oils and fats with low solubility have been investigated as carbon and energy sources for an effective antibiotic production. These are generally insoluble in a fermentation medium, which suggests that a practical means of avoiding catabolite repression is the use of vegetable oils as carbon sources. 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 [25]. Choi *et al.* [6] 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 tylosin production when rapeseed oils were used compared with using starch and glucose at the same initial concentration. In order to increase the oxygen transfer coefficient in the culture of tetracycline-producing *Streptomyces aureofaciens* using an

air-lift bioreactor, various vegetable oils and hydrocarbons were used. In the case of soybean oil or dodecan addition, the oxygen transfer coefficient was the highest [14].

Recently, we found that *Streptomyces lincolnensis* L1245 consumed vegetable oils as the sole carbon source and produced a large amount of lincomycin. 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 laundried clothes, and for impregnating paper and fabrics. It has also been useful for suppressing the growth of sensitive organisms in plate assays, and other biological media [17].

In this study, for efficient production of lincomycin from *Streptomyces lincolnensis* L1245, various vegetable oils, natural nitrogen sources, and surfactants were investigated at the pilot-scale level in the flask. After obtaining these results, fed-batch cultures for comparing the use of olive oil as the sole carbon source and starch as the conventional carbon source were carried out in a jar fermentor. Additionally, the correlation between the consumption rate of oil and lincomycin production in a culture using olive oil as the sole carbon source was investigated.

MATERIALS AND METHODS

Microorganism, Media, and Culture Conditions

The strain used in this study was *Streptomyces lincolnensis* L1245. The composition of the agar slant medium was as follows (g/l): glucose, 5; starch, 5; peptone, 2.5; yeast extract, 10; $MgSO_4 \cdot 7H_2O$, 1. The composition of the first seed medium used in the seed culture was as follows (g/l): starch, 20; yeast extract, 5; peptone, 5; $NaNO_3$, 0.5; $MgSO_4 \cdot 7H_2O$, 0.5. 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 or jar fermentor, the following medium was used (g/l); olive oil, 20; pharmamedia, 5; soybean meal, 5; KOH, 0.5; K_2HPO_4, 0.25; $MgSO_4 \cdot 7H_2O$, 0.5; trace elements solution, 3 ml. Trace elements solution contained the following ingredients (ppm): $FeCl_3$, 500; $ZnCl_2$, 600; $MnCl_2$, 100; $CoCl_2$, 300.

All media components were sterilized at 121°C and 1.2 atm for 20 min. The pH of the media was adjusted to 6.9 before sterilization. *Streptomyces lincolnensis* L1245 was transferred to the slant medium and cultured at 28°C for 7 days. Then, one loopful of the slant culture of *Streptomyces lincolnensis* L1245 was inoculated into a 500-ml Erlenmeyer flask containing 50 ml of the first seed medium and cultured at 28°C for 1 day on a reciprocating shaker at 120 rpm. For second seed, 2.5% of the first seed was inoculated into a 500-ml Erlenmeyer flask containing 50 ml of the second seed medium and cultured at 28°C for 1 day on a reciprocating shaker at 120 rpm. For production of lincomycin, 5% of the second seed was inoculated into a 500-ml Erlenmeyer flask containing 50 ml of the production medium or into a 5-l jar fermentor containing 2 l of production medium, and cultured at 28°C.

Cell Concentration

The cell concentration was determined by the intracellular nucleic acid (INA) concentration by Schneider's method [23], because the natural nitrogen source such as soybean meal, gluten meal, fish meal, and pharmamedia contained insoluble components that do not dissolve completely in the fermentation broth, and hence it is not useful to use the optical density or dry cell weight.

Oil Concentration

The oil concentration was measured by a solvent extraction method [6]. Three milliliters of culture broth was mixed with 6 ml of n-hexane and the mixture was vigorously shaken for 2 min in a capped Erlenmeyer flask, followed by centrifugation at 3,000 rpm for 15 min. The upper hexane layer was removed and dried at 80°C for 3 h, and the residue was weighed to determine the extracted oil weight.

Starch Concentration

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

Extracellular Lipase Assay

The lipase activity was determined titrimetrically as described by Behere *et al.* [2] and Song *et al.* [27]. The hydrolysis was performed in a 20 mM NaH_2PO_4-Na_2HPO_4 buffer, pH 7.5, at 37°C. The reaction was started by the addition of the enzyme preparation. The acid released was continuously titrated at pH 7.0 by a 0.5 M NaOH solution with pH-Stat. One unit of lipase activity was defined as the amount of enzyme that liberated 1 mol of free fatty acid per min under the above conditions.

Lincomycin Concentration

The lincomycin concentration was measured by the microbial assay using *Sareina lutea* ATCC 9341 as a test organism [13].

Apparent Viscosity

The apparent viscosity of the culture broth was measured using a vibration viscometer (VM-IA, Yamaich Denki Co. Ltd., Tokyo, Japan) at room temperature.

Table 1. Effects of various vegetable oils on the concentration of cell and lincomycin.

Carbon sources (20 g/l)	Cell concentration (g/l)	Lincomycin concentration (g/l)
Sesame oil	0.95	0.42
Rapeseed oil	1.03	0.73
Soybean oil	1.10	0.71
Olive oil	1.00	1.0
Cottonseed oil	1.07	0.54
Corn oil	0.92	0.7
Peanut oil	0.90	0.36

RESULTS

Effects of Various Vegetable Oils on the Lincomycin Production

In order to investigate the effect of various vegetable oils on the lincomycin production and cell concentration, sesame oil, rapeseed oil, soybean oil, olive oil, cottonseed oil, corn oil, and peanut oil as the sole carbon source were used. Batch culture was carried out in a flask containing 50 ml of production medium with 20 g/l of each carbon source for 5 days. The concentration of cell and lincomycin are shown in Table 1. When olive oil was used, the highest lincomycin concentration, 1.0 g/l, was obtained. The cell concentration was almost similar to that of other oils (0.9-1.1 g/l). In the case of apparent viscosity, it was also similar to that of other oils (data not shown). This indicated that olive oil is the most suitable carbon source for an efficient lincomycin production by *Streptomyces lincolnensis* L1245.

Effects of Olive Oil Concentrations on the Lincomycin Production

In order to determine the optimal initial concentration of olive oil for the effective lincomycin production, initial oil concentrations of 10, 20, 30, 40, and 50 g/l were used for 5 days in flasks. The results are shown in Table 2. An initial concentration of 20 g/l gave the highest lincomycin concentration and lipase activity, 1.01 g/l and 182 U/ml, respectively. The consumed concentration of olive oil was 11.7 g/l. When 10 g/l of olive oil was used, the olive oil was totally consumed. However, when 30, 40, or 50 g/l of olive oil was used, the consumed concentrations of olive oil were 9.2, 4.5, and 1.8 g/l, respectively. The lincomycin concentrations were 0.7, 0.4, and 0.15 g/l, respectively. In the case of cell concentration, it decreased with the increase of olive oil concentrations. In the case of lipase activity, when 20 g/l of olive oil was used, it was the highest. But, when approximately 20 g/l of olive oil was used, it deceased. This may be due to the production of some inhibitory by-product such as free fatty acids from olive oil. When the initial olive oil concentration was 20 g/l, the product yield was maximal at 0.086 g-lincomycin/g oil consumed.

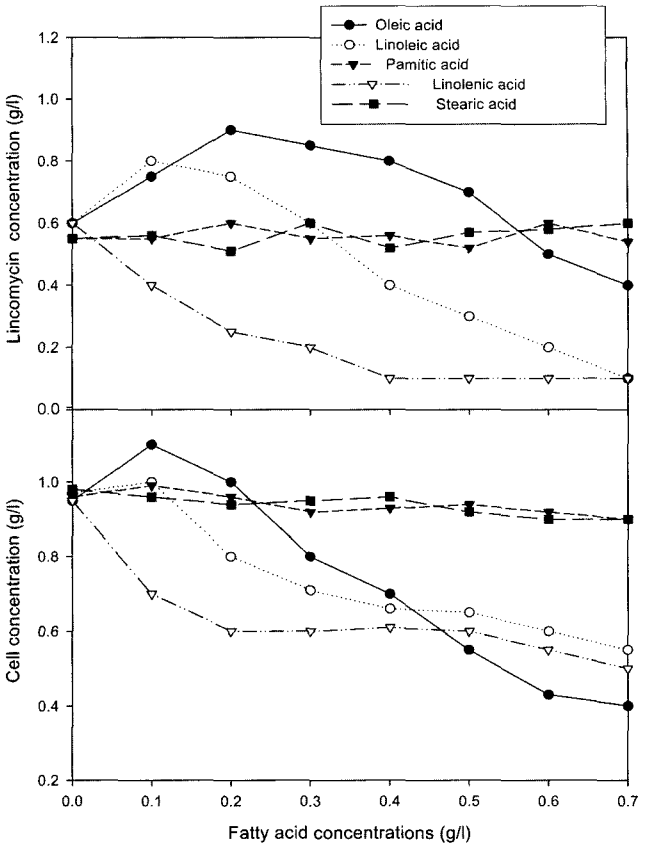

Fig. 1. Effect of various fatty acids on the lincomycin production and cell growth.

Effect of Each Unsaturated Fatty Acid Concentration on the Cell Growth and Lincomycin Production

In order to investigate the effect of each unsaturated fatty acid, which is decomposed by lipase from cells on the

Table 2. Effects of initial olive oil concentration on the concentration of cell, lincomycin, and residual olive oil, and lipase activity.

Olive oil concentration (g/l)	Cell concentration (g/l)	Lincomycin concentration (g/l)	Residual oil concentration (g/l)	Lipase activity (U/ml)
10	0.99	0.80	0	170
20	0.95	1.01	8.3	182
30	0.78	0.70	20.8	160
40	0.60	0.40	35.5	50
50	0.39	0.15	48.2	19

concentration of cell and lincomycin, oleic acid, linoleic acid, linolenic acid, palmitic acid, and stearic acid were used in flasks for 5 days. Each unsaturated fatty acid was added after 1 day of culture at the concentration of 0.1-0.7 g/l. The results are shown in Fig. 1. The cell growth of *Streptomyces lincolnensis* L1245 in medium containing palmitic acid or stearic acid was similar to that obtained without addition of palmitic acid or stearic acid. However, in the case of medium containing linolenic acid, it was markedly decreased. When 0.2 g/l of oleic acid was added to culture broth, the maximum lincomycin concentration was 1.0 g/l, which was about 1.7-fold higher than that obtained without the addition of oleic acid. In the case of addition of 0.1 g/l of linoleic acid, the maximum lincomycin concentration was 0.8 g/l. Palmitic acid and stearic acid concentration did not affect lincomycin production. This indicates that oleic acid is the most effective unsaturated fatty acid for the synthesis of lincomycin.

Effect of Various Nitrogen Sources on the Lincomycin Production

For investigating the effect of various natural nitrogen sources on the lincomycin production, cell growth, and apparent viscosity, peptone, yeast extract, pharmamedia, corn steep liquor (CSL), gluten meal, fish meal, and soybean meal were used. Batch culture was carried out in a flask containing 50 ml of production medium with 10 g/l of each natural nitrogen source for 5 days. The results are shown in Table 3. Among the various natural nitrogen sources, 10 g/l of pharmamedia or soybean meal was the most suitable nitrogen source. In particular, in the case of the mixture of 10 g/l of pharmamedia and soybean meal, 1.5 g/l of lincomycin concentration was produced and the cell concentrations ranged from 0.9 to 1.1 g/l. In addition, the consumed concentration of olive oil and lipase activity were increased. On the other hand, when yeast extract or peptone was used, cell concentration and apparent viscosity were the highest, but lincomycin concentration was only 0.2 g/l. This suggests that pharmamedia and soybean meal might contain components that are effective for lincomycin production.

Effect of Various Surfactants on the Lincomycin Production

In order to investigate the effects of various surfactants on the lincomycin production, cell growth, residual oil concentration, and lipase activity, Negamine 142-A, Span 180, Sodium dodecyl sulfate, Alrolene 65, Sunnix PP 4000, Triton X-100, Lecithin, and Tween 80 were used. Batch culture was carried out in a flask containing production medium with 0.3 g/l of each surfactant concentration for 5 days. The results are shown in Table 4. When Spane 180 was used, lincomycin concentration and lipase activity were highest at 1.85 g/l and 250 U/ml, respectively. The consumption of olive oil was increased more than that without addition of spane 180. The cell concentration ranged from 0.89 to 1.12, except for Tween 80. Apparent viscosity was also similar to that of other surfactants, except for Tween 80 (data not shown).

Comparison Between Olive Oil and Starch as a Carbon Source for Lincomycin Production

In order to compare the use of olive oil and starch on the lincomycin production, fed-batch culture was carried out in a 5-l jar fermentor containing 2 l of production medium for 7 days. The cell growth, carbon source concentration, apparent viscosity, and lincomycin production are shown in Fig. 2. The feeding of olive oil and starch was carried out after 2 and 4 days of culture, respectively. With a mixture of pharmamedia and soybean meal, the feeding was carried out after 3 days of culture. The DO

Table 3. Effects of various nitrogen sources on the concentration of lincomycin, cell, and residual oil; apparent viscosity; and lipase activity.

Nitrogen sources (g/l)	Cell con. (g/l)	Lincomycin con. (g/l)	Residual oil con. (g/l)	Apparent viscosity (cP)	Lipase activity (U/ml)
Soybean meal (10)	1.04	1.02	8.4	150	180
Pharmamedia (10)	1.05	1.25	5.0	110	195
Pharmamedia (5)+Soybean meal (5)	0.98	1.50	3.3	108	220
CSL (10)	1.00	0.98	10.0	98	170
CSL (5)+Soybean meal (5)	0.90	1.1	7.4	120	190
Gluten meal (10)	0.67	0.54	14.2	87	100
Gluten meal (5)+Soybean meal (5)	0.90	0.78	12.1	112	150
Fish meal (10)	0.90	0.56	14.8	88	106
Fish meal (5)+Soybean meal (5)	1.05	0.60	13.5	100	110
Peptone (10)	1.48	0.14	18.3	172	28
Peptone (5)+Soybean meal (5)	1.22	0.34	16.5	134	55
Yeast extract (10)	1.67	0.20	17.7	187	40
Yeast extract (5)+Soybean meal (5)	1.31	0.15	17.9	152	33

Table 4. Effects of various surfactants on the concentration of lincomycin and cell.

Surfactants (0.3 g/l)	Cell concentration (g/l)	Lincomycin concentration (g/l)	Residual oil concentration (g/l)	Lipase activity (U/ml)
Negamine 142-A	0.89	1.0	8.0	185
Span 180	1.05	1.85	0.7	250
Sodium dodecyl sulfate	1.12	0.8	10.2	160
Alrolene 65	0.94	0.5	14.0	95
Sunnix PP 4000	1.15	1.1	7.5	187
Triton X-100	1.10	1.02	8.4	190
Lecithin	1.05	1.35	5.0	210
Tween 80	0.79	0.3	16.0	55

concentration was similar to that of other carbon sources because the oxygen-enriched air was supplied in order to maintain DO concentration at 20-30% throughout the experiment with an automated oxygen concentrator. When olive oil was used as the sole carbon source, 34 g/l of olive oil were consumed after 7 days of culture. The maximum lincomycin concentration was 3.0 g/l after 7 days of culture. The apparent viscosity ranged from 80 to 100 cP after a culture of 2 days. The cell concentration ranged from 0.9 to 1.1 g/l after 2 days of culture. On the other hand, In the case of starch medium, 55 g/l of starch were consumed, but the produced maximum lincomycin concentration was 1.5 g/l after 7 days of culture. The product yield from olive oil and starch were 0.09 and 0.03 g/g consumed carbon source, respectively. Apparent viscosity was maximal at 120 cP at a culture time of 2 to 4 days and then decreased. The product yield coefficients for the other vegetables oils ranged from 0.06 to 0.076 g/g in flask cultures. In some cases of industrial antibiotic production, starch has been used as a carbon source. However, in the case of lincomycin production by *Streptomyces lincolnensis* L1245, the yield using olive oil was markedly higher than that of starch. This indicates that olive oil is the most suitable carbon source for the efficient production of lincomycin from *Streptomyces lincolnensis* L1245.

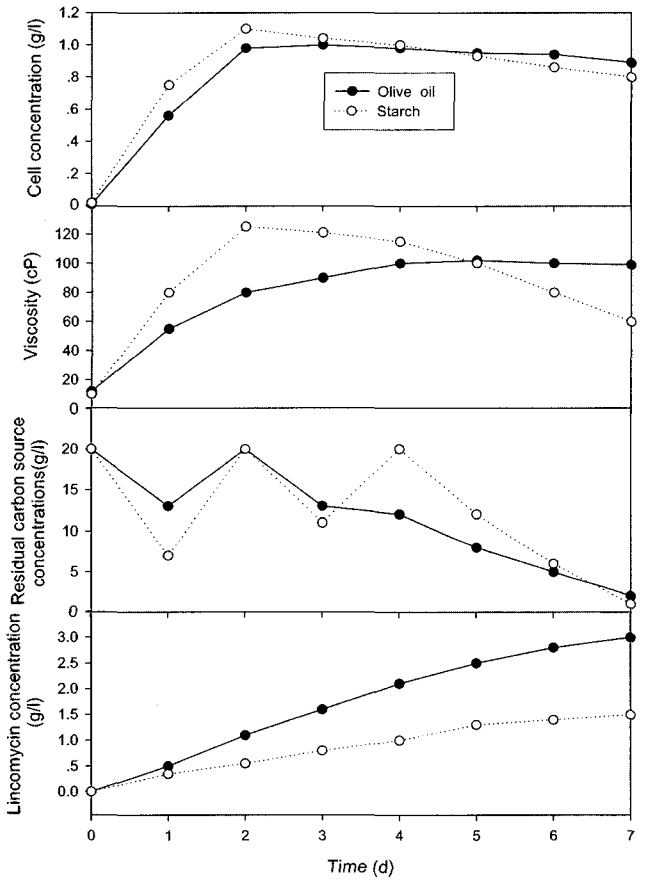

Fig. 2. Comparison of olive oil and starch on the lincomycin production, cell growth, carbon source concentration, and apparent viscosity.

DISCUSSION

Vegetable oils such as olive oil, cottonseed oil, soybean oil, rapeseed oil, peanut oil, and corn oil contained approximately

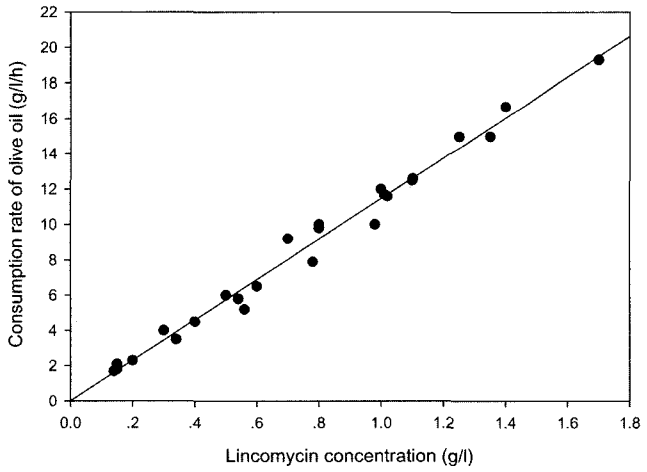

Fig. 3. Relationship between lincomycin production and consumption rate of olive oil.

2-4-fold the energy source as of glucose or sucrose on a 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 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 the volume of starch [1]. Oils are often cheap carbon sources compared to carbohydrates such as glucose and sucrose and are excellent product extractors. Eiki *et al.* [9] reported that they succeeded in producing 5-fold of josamycin by using an oily carbon source since josamycin distributed in the oily phase of the culture broth could be easily removed. Pan *et al.* [20] 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. Furthermore, various lipids including vegetable oils, long chain fatty acid, and animal oil as carbon sources for the efficient production of kasugamycin by *Streptomyces kadugaensis* were investigated [29]. Paul *et al.* [21] reported a 54% higher cephalosporin C production when 20 g/l of sesame oil was added to the culture broth using *Cephalosporium acremonium*. In the case of lincomycin production by *Streptomyces lincolnensis*, among the various vegetable oils, olive oil was the best carbon source for producing the lincomycin. When the corn oil, soybean oil, or rapeseed oil was used, the maximum lincomycin concentration was about 0.7 g/l. On the other hand, when glycerol was used as the sole carbon source, the cell concentration was about 1.5-fold higher than that of olive oil. However, the maximum lincomycin concentration was only 0.2 g/l (data not shown). In particular, when 20 g/l of olive oil was used, lincomycin production and lipase activity were the highest of all. However, when olive oil concentration above 40 g/l was used, lincomycin production, cell growth, lipase activity, and oil consumption were decreased. This may be due to the production of some inhibitory byproduct from oil.

Oil decomposed to glycerol and unsaturated fatty acids such as oleic, linoleic acid, and linolenic acid produced by cells. Therefore, the decomposed unsaturated fatty acid may be partially consumed by cells, and if a large amount is accumulated in the fermentation broth, they may act as an inhibitor for lincomycin production and cell growth. As shown in Fig. 1, among the unsaturated fatty acids, when linoleic acid and linolenic acid were used at higher than 0.3 and 0.1 g/l, respectively, cell growth and lincomycin production were decreased. On the other hand, in the case of oleic acid, they were decreased at 0.6 g/l. These results were similar to those of tylosin-producing *Streptomyces fradiae* T 1558 [5]. As a consequence, in order to improve the lincomycin production from oils, it is necessary to obtain a strain resistant to a high concentration of unsaturated fatty acids.

Regarding antibiotic production in actinomyctes, many authors have reported that the type and concentration of various nitrogen sources in a growth medium have some influence [3, 12]. Choi *et al.* [7] previously applied the natural nitrogen sources such as gluten meal and pharmamedia in an air-lift bioreactor for the effective tylosin production from *Streptomyces fradiae* T 1555 because the natural nitrogen sources contained various amino acids. These were also a cheap and commercially available source in the fermentation process. In the case of lincomycin production by *Streptomyces lincolnensis*, the highest lincomycin was produced when pharmamedia or soybean meal was used as the sole nitrogen source. In particular, in the case of the mixture of pharmamedia and soybean meal, the lincomycin production was increased more than each used alone as a sole nitrogen source. The apparent viscosity in the culture broth was similar although the pharmamedia or soybean meal concentration was increased as the sole nitrogen source (data now shown). This result was similar to that of tylosin-producing *Streptomyces fradiae* T 1558 [5]. On the other hand, in the case of the mixture of pharmamedia and soybean meal, the apparent viscosity was increased with the increase of the mixture concentration. However, lincomycin production and oil consumption were decreased (data now shown).

Many surfactants have been used with microorganism cultures for a number of different applications. For example, nonionic surfactants were used to remove surface proteins and solubilize the bacterial membranes [4]. In the case of the polyoxyethylenenonyl phenol ether, it was used as the antisporulating reagent for the effective production of AICA-riboside during the culture of *Bacillus megaterium* MA-336 [15]. Lonvaud-Funel *et al.* [16] reported that the presence of Tween 80 in the growth medium induced marked differences in the fatty acid composition of *Oenococcus oeni*. Choi *et al.* [6] previously used the Lecithin for the effective tylosin production when rapeseed oil was used as the sole carbon source in the culture of *Streptomyces fradiae* T 1555. This result indicated that the tylosin production was increased with the increase of rapeseed oil consumption by using surfactant in the production medium. In the case of lincomycin production using *Streptomyces lincolnensis*, among the various surfactants, the lincomycin production was higher than that of the other surfactants when Span 180 was used. In addition, the consumed concentration of olive oil was increased more than that without addition of spane 180. The correlation between the consumption rates of oil and lincomycin production was also investigated in culture using olive oil as the sole carbon source. As shown in Fig. 3, the lincomycin production depended on the consumption rate of olive oil.

Based upon the results, the fed-batch cultures were carried out for comparing the use of olive oil and starch as

the conventional carbon source in a jar fermentor. When olive oil was used as the sole carbon source, 34 g/l of olive oil was consumed after 7 days of culture. The lincomycin production rate was 0.018 g/l/h, which was about 2.0-fold higher than that of starch medium after 7 days of culture. In the case of product yield, it was 0.09 g/g consumed carbon source, which was about 3.0-fold higher than that of starch medium after 7 days of culture.

We are now in the process of screening mutants that are resistant to high concentrations of unsaturated fatty acids. Moreover, we have been 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. If fish oils can be used as a carbon source, the product costs of lincomycin will be drastically reduced.

Acknowledgments

We thank Professor Shiru Jia, Department of Biochemical Engineering, Tianjin University of Science and Technology, China, and Professor Young Soo Park Biochemical Engineering Lab, Applied Biological Chemistry, Shizuoka University, Japan, for their research advice.

REFERENCES

1. Bader, F. G., M. K. Boekeloo, H. E. Graham, and J. W. Cagle. 1984. Sterilization of oils: Data to support the use of a continuous point-of-use-sterilizer. *Biotech. Bioeng.* **26:** 848-856.
2. Behere, A. S., S. S. Dighe, S. B. Bhosale, and D. R. Ranade. 2002. Purification and characterization of lipase from the anaerobic lipolytic bacterium *Selenomonas lipolytica*. *J. Microbiol. Biotechnol.* **12:** 142-144.
3. Benslimane, C., A. Lebrihi, A. Lounes, G. Lefebver, and P. Germain. 1995. Influence of dextrins on the assimilation of yeast extract amino acid in culture of *Streptomyces ambafaciens* producer of spiramycin. *Enzyme Microb. Technol.* **17:** 1003-1013.
4. Chang, H. W. and E. Bock. 1980. Pitfalls in the use of commercial nonionic detergents for the solubilization of integral membrane proteins: Sulfahydryl oxidizing contaminants and their elimination. *Anal. Biochem.* **104:** 112-117.
5. Choi, D. B. 1998. Studies on efficient tylosin production from actinomyces using vegetable oil as the sole carbon source. Doctor thesis. Gifu Unversity, Japan.
6. Choi, D. B., S. Tamura, Y. S. Park, M. Okabe, Y. Seriue, and S. Takeda. 1996. Efficient tylosin production from *Streptomyces fradiae* using rapeseed oil. *J. Ferment. Bioeng.* **82:** 183-186.
7. Choi, D. B., Y. S. Park, and M. Okabe. 1998. Improvement of tylosin production from *Streptomyces fradiae* culture by decreasing the apparent viscosity in an air-lift bioreactor. *J. Fermet. Bioeng.* **86:** 413-417.
8. Dubois, M., K. A. Gilles, J. K. Hamilton, P. A. Rober, and F. Smith. 1956. Calorimetric method for determination of sugars and related substances. *Anal. Chem.* **28:** 350-356.
9. Eiki, H., H. Gushima, T. Saito, H. Ishida, Y. Oka, and T. Osono. 1988. Product inhibition and its removal on josamycin fermentation by *Streptomyces narbronesis var josamyceticus*. *J. Ferment. Technol.* **66:** 559-565.
10. Escalante, L., H. Lopez, R. C. Mateos, F. Lara, and S. Sanchez. 1982. Transient repression of antibiotic formation in *Saccharopolyspora erythraea*. *J. Gen. Microbiol.* **128:** 2011-2015.
11. Flores, M. E. and S. Sanchez. 1989. Ammonium assimilating enzymes and erythromycin formation in *Saccharopolyspora erythraea*. *J. Gen. Microbiol.* **35:** 203-211.
12. Flores, M. E. and S. Sanchez. 1985. Nitrogen regulation of erythromycin formation in *Streptomyces erythreus*. *FEMS Microb. Lett.* **26:** 191-194.
13. Hanka, L. J., D. J. Mason, M. R. Burch, and R. W. Treick. 1963. Lincomycin, a new antibiotic. II. Microbial assay. *Antimicrob. Agents Chemother.* 1962. 565-569.
14. Jia. S., G. Chen, K. Prihardi, D. B. Choi, and M. Okabe. 1999. Effect of soybean oil on oxygen transfer in the production of tetracycline with an airlift bioreactor. *J. Biosci. Bioeng.* **87:** 825-827.
15. Kinoshita, K., S. Sada, M. Yasunaga, and T. Shiro. 1969. Studies on the fermentation production of AICA-riboside. *Nippon Nogeikagaku Kaishi* **43:** 404-409.
16. Lonvaud-Funel, A. and C. Desens. 1990. Constitution en acids gras des membranes des bacteries lactiques du vin: Incidences des conditions de culture. *Sciences des Aliments.* **10:** 817-829.
17. Malcolm, E. B. and R. H. Ross. 1963. Antibiotic lincomycin and method of production. *USA patent*, 3,086,912.
18. Martin, J. F. and A. L. Deman. 1980. Control of antibiotic biosynthesis. *Microbiol. Rev.* **44:** 230-251.
19. Martin, J. F., G. Revilla, D. M. Zanca, and M. J. Lopez-Nieto. 1980. Carbon catabolite regulation of penicillin and cephalosporin biosynthesis, pp. 258-268. *In* Umezawa, H. *et al.* (eds.) *Trends in Antibiotic Research.* Japan Antibiotic Research Association Press, Tokyo.
20. Pan, C. H., S. V. Speth, E. Mckillip, and C. H. Nash. 1982. Methyl oleate-base medium for cepalosporin C production. *Dev. Ind. Microbiol.* **23:** 315-323.
21. Paul, S., R. L. Bezbaruah, R. S. Prakasham, M. K. Roy, and A. C. Ghosh. 1997. Enhancement of growth and antibiotic titer in *Cephalosporium acremonium* induced by sesame oil. *Folia Microbial* **42:** 211-213.
22. Potvin, J. and P. Peringer. 1994. Ammonium regulation in *Saccharopolyspora erythraea*. I: Growth and antibiotic production. *Biotechnol. Lett.* **16:** 63-68.
23. Schneider, W. 1945. Phosphorous compounds in animal tissues. I. Extraction and estimation of deoxypentose nucleic acid. *J. Biol. Chem.* **161:** 293-295.
24. Sohng, J. K., H. C. Lee, K. K. Liou, E. B. Lee, S. Y. Kang, and J. S. Woo. 2003. Cystocin, a novel antibiotic,

produced by *Streptomyces* sp. GCA0001: Production and characterization of cystocin. *J. Microbiol. Biotechnol.* **13:** 483-486.
25. Soltero, F. V. and M. J. Johnson. 1953. Effect of the carbohydrate nutrition on penicillin production by *Penicillium chrysogenum* Q-176. *Appl. Microbiol.* **1:** 52-57.
26. Soltero, F. V. and M. J. Johnson. 1954. Continuous addition of glucose for evaluation of penicillin-production cultures. *Appl. Microbiol.* **2:** 41-44.
27. Song, X., Q. Yinbo, D. H. Shin, and E. K. Kim. 2001. Purification and characterization of lipase from *Trichosporon* sp. Y-11 and its use in ester synthesis of unsaturated fatty acid and alcohols. *J. Microbiol. Biotechnol.* **11:** 951-956.
28. Trilli, A., M. V. Crossley, and M. Kontakou. 1987. Relation between growth rate and erythromycin production in *Streptomyces erythraeus*. *Biotechnol. Lett.* **9:** 765-770.
29. Yagi, Y., I. Kitamura, K. Okamura, A. Ozaki, M. Hamada, and H. Umezawa. 1971. Production of kasugamycin by *Streptomyces kadugaensis*. *J. Ferment. Technol.* **49:** 117-127.