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Tylosin Production by *Streptomyces fradiae* Using Raw Cornmeal in Airlift Bioreactor

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Abstract Using a 50-l airlift bioreactor, for the effective production of tylosin from *Streptomyces fradiae* TM-224 using raw cornmeal as the energy source, various environmental factors were studied in flask cultures. The maximum tylosin concentration was obtained at 32°C and pH between 7.0 and 7.5. When seed was inoculated after 24 h of culture, the maximum tylosin concentration, 5.7 g/l, was obtained after 4 days of culture. Various concentrations of raw cornmeal were tested to investigate the optimum initial concentration for the tylosin production. An initial raw cornmeal concentration of 80 g/l gave the highest tylosin concentration, 5.8 g/l, after 5 days of culture. Of the various nitrogen sources, soybean meal and fish meal were found to be the most effective for the production of tylosin. In particular, with the optimal mixing ratio, 12 g/l of soybean meal to 14 g/l of fish meal, 7.2 g/l of tylosin was obtained after 5 days of culture. To compare raw cornmeal and glucose for the production of tylosin in the 50-l airlift bioreactor for 10 days, fed-batch cultures were carried out under the optimum culture conditions. When raw corn meal was used as the energy source, the tylosin production increased with increasing culture time. The maximum tylosin concentration after 10 days of culture was 13.5 g/l, with a product yield from raw cornmeal of 0.123 g/g of consumed carbon source, which was about 7.2 times higher than that obtained when glucose was used as the carbon source.

Keywords: Tylosin, raw cornmeal, *Streptomyces fradiae*, airlift bioreactor

Cornstarch has been used as the energy sources for producing sugars in the fermentation industry for a long time. Ethanol and lactic acid have been effectively produced *via* the enzymatic hydrolysis of cornstarch by microorganisms [13, 22, 26]. Jun *et al.* [18] reported the production of lactic acid by simultaneous saccharification and fermentation from an acid-hydrolyzed corncob using a cellulose preparation and *Lactobacillus delbrueckii*. Yin *et al.* [34] also reported the optimal conditions for the production of lactic acid using cornstarch sugars in both flask and airlift bioreactor cultures of *Rhizopus oryzae* NRRL 359. In the case of *Candida albicans*, various mixtures containing cornmeal were used as the energy source for chlamydospores production. Specifically, the production of chlamydospore was most effective in the medium containing 3% of cornmeal plus 5% of milk serum [1, 25]. For alpha-amylase production in the solid culture using *Hericium erinaceum* and *Ganoderma lucidum* and in the liquid culture using *Drosophila melanogaster*, *D. funebris*, and *D. saltans*, cornmeal has been used [11, 14, 15]. The corn fiber xylan was also used in order to produce an acetone, butanol, and ethanol from *Clostridium acetobutylicum* P260 [31]. Jia *et al.* [17] previously used a mixture of cornstarch and soybean oil in the effective production of tetracycline from *Streptomyces aureofaciens* CG-1. The tetracycline production increased by the increase of oxygen transfer rate in the culture broth. However, it was expensive and difficult to produce sugars from raw corn, and there was also some problem to decrease the amount of waste corn-sludge in the process of cornmeal manufacture. Therefore, we tried to use raw cornmeal in the fermentation industry without pretreatment of the raw corn. Raw cornmeal is a combined carbon and nitrogen source, containing about 10–11% protein, 5–6% corn oil, and 70–72% starch. We are currently attempting to confirm the possibility of enhancing the production of antibiotic using an airlift bioreactor, because they have many economical implications with regard to reactor construction, maintenance, and scale-up, as previously reported [5, 17]. However, the ability of an airlift bioreactor to supply oxygen

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is generally poorer than that of conventional bioreactors, *i.e.*, aeration and stirred tank bioreactors. Therefore, it is necessary to find the optimal culture conditions suitable for the operation of an airlift bioreactor. Recently, we found that tylosin could be produced from cornstarch in a culture of *Streptomyces fradiae*.

In this study, for the effective production of tylosin in a culture of *Streptomyces fradiae* TM-224, raw cornmeal was used in place of other energy sources; firstly, media and cultivation conditions were optimized in a flask cultures. Secondly, the feasibility of tylosin production in fed-batch cultures using a 50-l airlift bioreactor employing the optimum results was investigated.

MATERIALS AND METHODS

Strain, Media, and Culture

The tylosin-producing strain used in this study was *Streptomyces fradiae* TM-224. The composition of the agar medium was as follows (g/l): cornstarch, 10; yeast extract, 5; $MgSO_4 \cdot 7H_2O$, 0.5; NaCl, 0.5; and agar, 10. The composition of the seed medium was as follows (g/l): corn oil, 1; cornstarch, 5: yeast extract, 1; soybean meal, 2; $NaNO_3$, 0.5; and $MgSO_4 \cdot 7H_2O$, 0.5. For the production of tylosin, the basal medium used was as follows (g/l): K_2HPO_4, 0.25; $MgSO_4 \cdot 7H_2O$, 0.5; and a solution of trace elements, 3 ml. The trace element solution contained the following ingredients (ppm): $FeCl_3$, 500; $ZnCl_2$, 600; $MnCl_2$, 100; $CoCl_2$, 300. The pH of the media was adjusted to 7.0–7.5 using 2 M KOH prior to sterilization. All the media components were sterilized at 121°C and 1.2 atm for 30 min. In the case of cornstarch, it was partially hydrolyzed by the addition of 0.2% alpha-amylase (HS, Nagase Biochem. Ind. Ltd., Kyoto, Japan) at 121°C for 30 min. One loopful of *Streptomyces fradiae* TM-224 was transferred to a slant of the medium and cultured for 5 days. One loopful of the slant culture of *Streptomyces fradiae* TM-224 was then inoculated into a 500-ml Erlenmeyer flask, containing 50 ml of the seed medium, and cultured for one day on a reciprocating shaker at 120 rpm. For the production of tylosin, 10% of the seed was inoculated into a 500-ml Erlenmeyer flask, containing 50 ml of the production medium, or to an airlift bioreactor, and cultured at 32°C.

Cell, Antibiotic, and Carbon Sources Concentration

The cell, antibiotic, and carbon sources concentrations were measured using a modification to the methods of Kim *et al.* [20], Ei-Nagga *et al.* [10], and Kim *et al.* [21], respectively.

Extracellular Lipase and Alpha-Amylase Assay

The activities of lipase and alpha-amylase were measured using a modification to the methods of Park *et al.* [28, 29] and Silva *et al.* [33], respectively.

Apparent Viscosity

The apparent viscosity was measured using a vibration type viscometer (VM-IA; Yamaichi Electric, Tokyo) at room temperature.

Airlift Bioreactor System

Based on the designs described elsewhere [19, 23, 30], a 50-l airlift bioreactor was modified for the culture of *Streptomyces fradiae* TM-224. The airlift bioreactor comprised three parts; a conical bottom holding the sparger, a cylindrical middle section, and a top portion with a degassing zone. In order to increase the mixing characteristics, the draft tube was removed and replaced with four ring spargers and wire nets. An airlift bioreactor has several ports; for measuring the dissolved oxygen concentration and foam inside the bioreactor, for the removal of exhaust gas, and for the addition of antifoam agents and feeding medium, with a sampling port at the bottom of the cylindrical section. The temperature of the contents of an airlift bioreactor can be controlled by circulating water through a jacket. In this experiment, the airlift bioreactor was operated for 10 days.

RESULTS

Comparison of *Streptomyces fradiae* Strains on the Concentration of Tylosin and Its Analogs

In order to compare the tylosin production from *Streptomyces fradiae*, various strains from oleic acid mutants were tested for 3 day in flasks at 210 rpm using a basal medium containing 60 g/l of raw cornmeal. Table 1 shows the results obtained from six representative strains. All the strains produced antibiotics, either macrocin or relomycin. Of the strains tested, the macrocin concentration was 1.9 g/l

Table 1. Comparison of *Streptomyces fradiae* strains on tylosin, cell, and tylosin analog concentrations.

Strains	Macrocin concentration (g/l)	Relomycin concentration (g/l)	Tylosin concentration (g/l)	Lipase activity (U/ml)	Alpha-amylase activity (U/ml)
TM-224-1	0.9	0.0	3.0	63.8	75.6
TM-224-2	0.4	0.6	1.4	23.5	36.7
TM-224-3	0.5	1.0	2.6	75.2	63.4
TM-224-4	0.3	0.1	3.8	82.3	110.5
TM-224-5	0.6	0.1	2.2	51.0	69.8
TM-224-6	0.6	1.0	3.3	78.1	98.4

Table 2. Effects of temperature on tylosin, cell, and residual carbon source concentrations.

Culture temperature (°C)	Cell concentration (g/l)	Residual corn oil concentration (g/l)	Residual cornstarch concentration (g/l)	Tylosin concentration (g/l)
28	0.78	3.0	23.9	3.1
30	0.92	0.7	18.5	4.0
32	0.95	0.5	5.8	4.7
34	0.97	1.2	11.8	3.6
36	0.80	2.5	29.7	3.0

in the *Streptomyces fradiae* TM-224-2, but the tylosin concentration was only 1.4 g/l. The relomycin production was the highest in *Streptomyces fradiae* TM-224-3 strain. Conversely, when the TM-224-4 strain was used, the concentration of tylosin was 3.8 g/l, corresponding to an approximate 2.7-fold, compared with the *Streptomyces fradiae* TM-224-2 strain. Specifically, when *Streptomyces fradiae* TM-224-4 was used, the activities of lipase and alpha-amylase were 82.3 and 110.5 U/ml, and about 3.5- and 3.0-fold higher than those of the TM-224-2 strain, respectively. The cell concentrations of *Streptomyces fradiae* TM-224-4 ranged from 0.85 to 0.95 g/l (data not shown), and therefore, it was used in the following experiments.

Effect of Culture Temperature on Cell Concentration and Tylosin Production

In order to investigate the effects of temperature on the cell concentration and tylosin production, batch cultures maintained at various temperatures were carried out for 3 days using *Streptomyces fradiae* TM-224-4. The culture temperatures varied within the range of 28–36°C at intervals of 2°C. The results are shown in Table 2. The cell concentrations in the culture between 30 and 34°C were within the range of 0.92 and 0.97 g/l, but in the cases of cultures above 36 or below 28°C, the cell concentrations decreased. The consumptions of corn oil and cornstarch increased with increasing culture temperature up to 32°C, but decreased at temperatures above 34°C. The tylosin production generally increased with increasing culture temperature up to 32°C, reaching 4.7 g/l, but decreased at temperatures above 34°C. This finding indicates that the production of tylosin in the *Streptomyces fradiae* TM-224-4 culture was significantly affected by the culture temperature.

Effect of pH on Cell Concentration and Tylosin Production

In order to investigate the effects of initial medium pH on the cell concentration and tylosin production, batch cultures were carried out within the pH ranging from 6.5 to 8.5 and the results are shown in Table 3. When the cultures were grown within the pH range of 7.0 to 7.5, the cell concentrations ranged from 0.90 to 0.91 g/l, but above pH 7.5 or below 7.0 they were decreased. The concentrations of residual corn oil and cornstarch ranged from 0.6 to 1.0 and 8.5 to 13.4 g/l, respectively, between pH 7.0 and 7.5. The optimum pH for effective production of tylosin ranged between 7.0 and 7.5. The maximum tylosin production at pH 7.5 was 4.8 g/l.

Effect of Preculture Age on Cell Concentration and Tylosin Production

To investigate the effects of inoculation time on the production of tylosin and the cell and carbon source concentrations, batch cultures were performed at 210 rpm for 4 days at various inoculation times in media containing 70 g/l of raw cornmeal. The cultures were carried out at inoculation times of 20, 24, 28, 32, and 36 h, and the results are shown in Table 4. The maximum tylosin concentration, 5.7 g/l, was obtained with 24 h of inoculation culture, and the consumed corn oil and cornstarch concentrations were 0.7 and 49.5 g/l, respectively. The cell concentration was similar to that of the other inoculation times, with the exception of 20 h of inoculation. When inoculations were carried out at 20, 28, 32, and 36 h of culture, the consumed corn oil and cornstarch concentrations were 1.8 and 40.6, 2.3 and 46.5, 2.3 and 43.2, and 1.6 and 39.6 g/l, respectively, and the tylosin concentrations were 3.5, 4.9, 4.1, and 3.8 g/l, respectively.

Table 3. Effects of pH on the tylosin, cell, and residual carbon source concentrations.

Initial pH	Cell concentration (g/l)	Residual corn oil concentration (g/l)	Residual cornstarch concentration (g/l)	Tylosin concentration (g/l)
6.5	0.62	2.6	22.3	3.0
7.0	0.91	1.0	13.4	4.7
7.5	0.90	0.6	8.5	4.8
8.0	0.79	2.7	12.3	4.1
8.5	0.69	3.5	19.5	2.8

Table 4. Effects of preculture age on tylosin, cell, and residual carbon source concentrations.

Preculture age (h)	Cell concentration (g/l)	Residual corn oil concentration (g/l)	Residual cornstarch concentration (g/l)	Tylosin concentration (g/l)
20	0.75	1.7	8.4	3.5
24	0.92	1.0	1.5	5.7
28	0.93	1.2	2.5	4.9
32	0.88	1.2	5.8	4.1
36	0.78	1.9	9.4	3.8

Effect of Raw Cornmeal Concentration on Cell Concentration and Tylosin Production

To elucidate the optimum initial concentration of raw cornmeal for the effective production of tylosin, initial concentrations of 50, 80, 110, 140, and 170 g/l were investigated. Batch cultures for 5 days were carried out using *Streptomyces fradiae* TM-224-4. The cell, residual carbon sources, and tylosin concentrations are shown in Table 5. When 50 g/l of raw cornmeal was used, the corn oil and cornstarch were perfectly consumed. However, when 110, 140, and 170 g/l of raw cornmeal were used, the consumed corn oil and cornstarch concentrations were 2.0 and 67.0, 2.0 and 61, and 1.5 and 48.7 g/l, respectively. The cell concentrations ranged between 0.8 and 0.89 g/l. In the case of tylosin, an initial raw cornmeal concentration of 80 g/l gave the highest tylosin concentration, 6.2 g/l.

Effect of Nitrogen Sources on Cell Concentration and Tylosin Production

To investigate the effects of various nitrogen sources on the production of tylosin, the residual carbon sources and cell concentrations, media containing peptone, yeast extract, gluten meal, fishmeal, soybean meal, ammonium sulfate, and urea were used. Batch cultures were carried out in flasks containing 50 ml of the basal medium and 5 g/l soybean meal and 80 g/l of raw cornmeal, with 10 g/l of one of the nitrogen sources for 5 days, and the results are shown in Table 6. Of the various nitrogen sources tested, the mixture of soybean meal or fish meal gave the highest tylosin concentration. The cell concentrations ranged from 0.83 to 0.89 g/l. Conversely, when yeast extract and peptone were used as the nitrogen sources, the highest cell concentration was obtained. However, the tylosin concentrations ranged from only 3.0 to 3.2 g/l. In the cases of ammonium sulfate and urea used as inorganic nitrogen sources, the cell concentrations ranged between 0.40 and 0.45 g/l, with less than 2.0 g/l of tylosin production. This suggests that soybean meal and fish meal might contain unique components that are effective for the production of tylosin. Therefore, soybean meal and fish meal were used as the nitrogen sources in the following experiments.

Effect of Mixing Ratio of Soybean Meal and Fish Meal on Cell Concentration and Tylosin Production

To determine the optimal mixing ratio of soybean meal and fish meal for obtaining a high level of tylosin production, batch cultures were carried out for 5 days in flasks. A total of 49 nitrogen sources were tested and the results are shown in Fig. 1. When the soybean meal concentrations were raised from 6 to 12 g/l, the tylosin concentration increased, but did not improve more than 14 g/l. The cell concentrations were not affected at soybean meal concentrations above 10 g/l (data not shown). When the fish meal concentration was raised from 6 to 14 g/l, the tylosin concentration increased, but also did not improve higher than 16 g/l. The cell concentrations also increased with increasing fish meal concentration (data not shown). The optimal mixing ratio was found to be 12 g/l of soybean meal to 14 g/l of fish meal, which gave a tylosin concentration of 7.4 g/l.

Comparison of Raw Cornmeal and Glucose on Cell Concentration, Carbon Source Consumption, Apparent Viscosity, and Antibiotics Production in a 50-l Airlift Bioreactor

Using the optimized culture conditions obtained above, for comparing the effects of raw cornmeal and glucose on the production of tylosin in the airlift bioreactor, fed-batch cultures were carried out in a 50-l airlift bioreactor containing

Table 5. Effects of raw cornmeal concentration on tylosin, cell, and residual carbon source concentrations.

Raw cornmeal concentration (g/l)	Cell concentration (g/l)	Residual corn oil concentration (g/l)	Residual cornstarch concentration (g/l)	Tylosin concentration (g/l)
50	0.80	0.0	0.0	5.6
80	0.89	0.5	0.0	6.2
110	0.85	3.0	10.0	5.8
140	0.86	5.0	36.8	4.1
170	0.80	6.5	70.3	2.0

Table 6. Effects of different nitrogen sources on tylosin, cell, and residual carbon source concentrations.

Nitrogen sources	Cell concentration (g/l)	Residual corn oil concentration (g/l)	Residual cornstarch concentration (g/l)	Tylosin concentration (g/l)
Gluten meal	0.83	1.0	4.0	6.1
Fish meal	0.86	0.2	1.5	6.7
Yeast extract	1.40	3.3	8.5	3.2
Soybean meal	0.89	1.5	5.5	6.3
Peptone	1.31	3.0	10.2	4.0
Ammonium sulfate	0.45	3.5	25.0	2.0
Urea	0.40	3.7	26.8	1.8

30 l of production medium for 10 days. The cell concentrations, carbon source concentrations, apparent viscosity, and production of tylosin are shown in Fig. 2. The feeding of raw cornmeal for the effective production of tylosin was carried out after 4 days of culture, and in the case of glucose, the feeding was carried out after 2 and 6 days of culture. When glucose was used as the carbon source, 180.0 g/l of glucose was consumed, but the maximum tylosin concentration, 3.1 g/l, was produced after 10 days of culture. The macrocin and relomycin concentrations increased with increasing culture time, with concentrations of 2.2 and 1.0 g/l, respectively, which were 11.0- and 10-fold higher than that of the raw cornmeal medium. The cell concentration was increased with increasing culture time up to 2 days, ranging from 1.25 to 1.5 g/l after 5 days of culture. However, after 6 days of culture, it began to decrease, finally reaching 0.90 g/l after 10 days of culture. The maximum apparent viscosity was 185 cP at 2 to 5 days of culture time, and decreased thereafter. Conversely, when

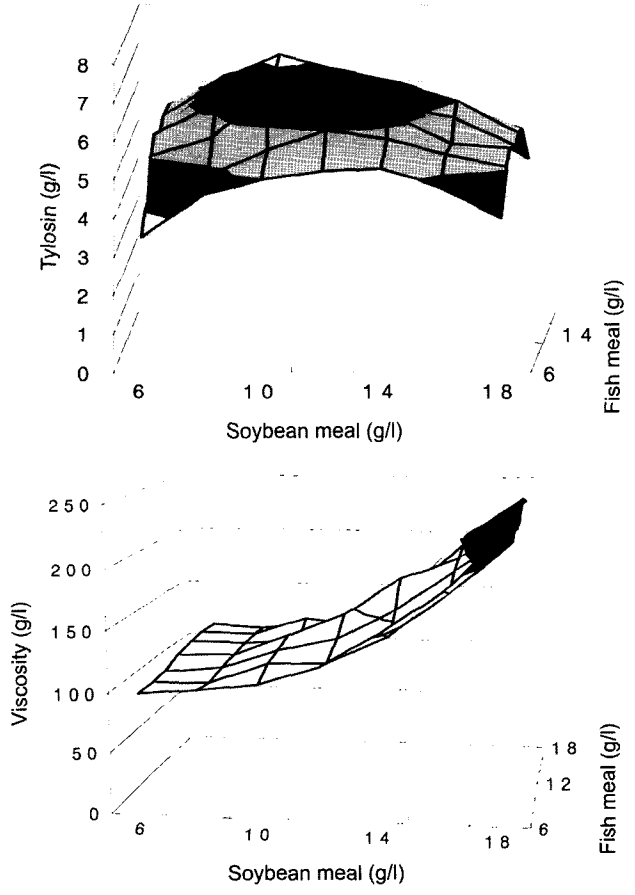

Fig. 1. Effects of soybean meal and fish meal mixing ratio on the production of tylosin and the apparent viscosity.

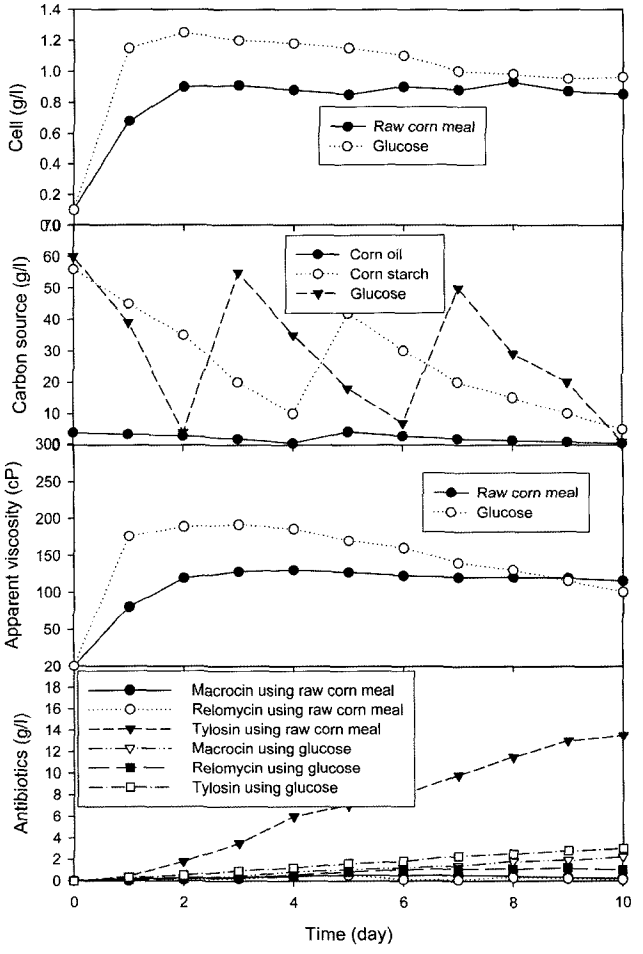

Fig. 2. Comparison of raw cornmeal and glucose on the cell concentration, carbon source consumption, apparent viscosity, and production of antibiotics in the air-lift bioreactor.

raw cornmeal was used as the carbon source, about 7.8 and 102.0 g/l of corn oil and cornstarch, respectively, were consumed after 10 days of culture. The production of tylosin increased with increasing culture time. The maximum tylosin concentration after 10 days of culture was 13.5 g/l, which was about 4.3-fold higher than that of the glucose medium. The apparent viscosity ranged from 117 to 121 cP after 2 days of culture. The cell concentrations ranged between 0.86 and 1.09 g/l after 2 days of culture.

DISCUSSION

Tylosin is derived from an inert polyketide lactone *via* glycosylation, with three different 6-deoxyhexose residues. The monsaccharide substituent at C_{23} of the polyketide lactone ring is added as 6-deoxyallose, which is subsequently converted to mycinose by *bis O*-methylation in the final two steps of tylosin biosynthesis. It is used in pharmaceuticals for the treatment of Gram-positive bacterial infections and mycoplasma, and shows especially strong antibacterial activity against *Pseudomonas aeruginosa* and *Salmonella typhimurium*. Moreover, it has been used as an intermediate of acetyl-isovaleryl tylosin [16]. The biosynthesis of tylosin and the regulation of its biosynthesis have been of interest to biochemists for more than a decade [5, 32]. Choi *et al.* [9] previously investigated various vegetable and animal oil, as the sole carbon source for the efficient production of tylosin in cultures of *S. fradiae* T1555. From this study, rapeseed oil was identified as effecting the productivity of tylosin, including the activity of methylmalonyl-CoA carboxyltransferase in the biosynthesis of the tylosin precursor, protylonolide, in a batch culture of *S. fradiae* T1555 [4]. Choi *et al.* [5] also investigated how to decrease the apparent viscosity of the *S. fradiae* culture broth, and reported that the apparent viscosity of the culture was dependent on morphological change of the mycelia. However, an explanation for the dependences of apparent viscosity has not yet been elucidated. Therefore, we investigated the relationship between the apparent viscosity and type of natural nitrogen source used in the culture of *S. fradiae* to find the reason for the decrease in the apparent viscosity. The extracellular protease activity increased with increases in the apparent viscosity and culture time, with the natural nitrogen source decomposed into amino acids; however, the apparent viscosity was not caused by the mycelia concentration, but by the mycelia forming filamentous morphology [6]. When high concentrations of vegetable oils were used in the culture media, the production of tylosin decreased owing to fatty acids, such as oleic acid, linoleic acid, and linolenic acid, from hydrolysis byproducts of vegetable oils [3]. Of these fatty acids, oleic acid was found to inhibit the growth of *Streptomyces fradiae*. Therefore, ultraviolet-induced mutation was carried out to obtain a mutant strain resistant to the fatty acids to improve the tylosin productivity. The cell growth was restrained by the addition of 0.8 g/l of oleic acid to the culture broth. A mutant TM-224 strain resistant to oleic acid was obtained by screening solid and liquid media containing oleic acid. However, it was difficult to use the refined vegetable oils as the carbon source in the effective tylosin production process because of its cost [8].

Recently, we observed the tylosin production from cornstarch in a *Streptomyces fradiae* TM-224 flask culture. In order to realize the objective of this research, various fatty acid mutants were tested in flask cultures. Of the strains tested, the concentration of tylosin was the highest in TM-224-4. Additionally, the activities of lipase and alpha-amylase were in proportion to the production of tylosin from *Streptomyces fradiae* TM-224-4. This suggests that relomycin and microsin, as tylosin analogs, were strongly affected by the activities of lipase and amylase produced from the *Streptomyces fradiae* TM-224 strain, which is resistant to oleic acid. Using *Streptomyces fradiae* TM-224, various environmental factors affecting the production of tylosin were researched in flask cultures. The maximum tylosin concentration was obtained at 32°C and between pH 7.0 and 7.5. Of the various amino acids, especially when cultures were performed at 32°C and between pH 7.0 and 7.5, threonine, serine, glutamic acid, alanine, valine, leucine, histidine, lysine, and tyrosine were consumed to a much greater extent than the other amino acids. On the other hand, the concentrations of glycine, methionine, phenylalanine, cystine, arginine, and aspartic acid consumed were similar to those of the cultures at other temperatures and pHs. This indicates that the production of tylosin was affected by threonine, serine, glutamic acid, alanine, valine, leucine, histidine, lysine, and tyrosine (data no shown).

To investigate the effects of inoculation time on the production of tylosin and cell and carbon sources concentrations, batch cultures were performed at various inoculation times. The maximum tylosin concentration was obtained with the culture at an inoculation time of 24 h, but this decreased when inoculations were carried out at either side of this time. However, the cell concentrations were similar to those of other inoculation times, with the exception of the 20 h of inoculation time. This indicates that the tylosin production of *Streptomyces fradiae* TM-224-4 was significantly affected by the inoculation time. Batch cultures were performed to find the optimum initial concentration of raw cornmeal for the effective production of tylosin using *Streptomyces fradiae* TM-224-4. When 50 g/l of raw cornmeal was used, corn oil and cornstarch were perfectly consumed. Specifically, when 80 g/l of raw cornmeal was used, the maximum tylosin concentration was obtained after 5 days of culture. This indicates that the tylosin production of *Streptomyces fradiae* TM-224-4 was significantly affected by the raw cornmeal concentration.

Nitrogen is one of the major components of living materials, and plays a key role in biological regulation. Nitrogen sources can govern cellular growth, formation of products, and cellular enzymes. The regulatory effects of nitrogen on the production enzymes involved in nitrogen assimilation, catabolism of nitrogenous compound, and formation of glutamate have been reviewed [24]. With respect to antibiotic production in *Actinomyctes*, many authors have reported that the type and concentration of various nitrogen sources in the growth medium have some influence [2, 12]. Choi *et al.* [7] previously applied nitrogen sources, such as soybean meal and pharmamedia, in an airlift bioreactor for the effective production of compactin from *Penicillium citrinum* L-18065, as these nitrogen sources contained various amino acids and are cheap and commercially available sources for fermentation processes. Therefore, various nitrogen sources were investigated for their effectiveness on the production of tylosin. Of the various nitrogen sources, the highest tylosin production was obtained when fish meal or soybean meal was used as the sole nitrogen source, with cell concentrations ranging from 0.83 to 0.89 g/l. On the other hand, the highest cell concentration was obtained when yeast extract and peptone were used, but tylosin concentrations were very low. When ammonium sulfate and urea were used as the inorganic nitrogen source, the cell concentrations were very low, with tylosin productions less than 2.0 g/l. This suggests that soybean meal and fish meal might contain unique components that are effective for tylosin production. When mixtures of fish meal and soybean meal were used, the production of tylosin was increased more than with a sole nitrogen source. When the fish meal concentration was increased, the apparent viscosities of the culture broth remained unchanged. In the culture used in this study, the apparent viscosity was similar to that of the culture of compactin-producing *Penicillium citrinum* L-18065 using fish meal medium [7]. On the other hand, in the case of the soybean meal medium, the apparent viscosity increased with increasing soybean meal concentration. This was similar to that of the cephamycin-producing *Streptomyces* sp. P6621 U-12-2 culture using soybean meal (data not shown).

Fed-batch cultures in a 50-l airlift bioreactor containing 30 l of production medium for 10 days were performed under the optimum culture conditions to compare raw cornmeal and glucose for the production of tylosin. When glucose was used as the carbon source, the macrocin and relomycin concentrations increased with increasing culture time, with concentrations 11.0- and 10-fold higher than those using raw cornmeal medium. This indicates that tylosin analogs are strongly affected by the carbon source. The maximum apparent viscosity was found with the 2 to 5 days of cultures, but decreased thereafter. The pH was 6.4 after 3 days of culture, but 8.0 after 10 days of culture (data not show). On the other hand, when raw cornmeal was used as the carbon source, about 7.8 and 102.0 g/l of corn oil and cornstarch, respectively, were consumed after 10 days of culture. The tylosin production increased with increasing culture time. The product yields from raw cornmeal and glucose were 0.123 and 0.017 g/g consumed carbon source, with the tylosin productions of the two cultures being 0.056 and 0.013 g/l/h, respectively. The pH was within the range of 6.5 to 7.5 throughout the entire culture (data not shown).

Glucose has been used as a carbon source in some cases of industrial antibiotic production. However, in the tylosin production by *Streptomyces fradiae* TM-224-4, the yield using raw cornmeal was markedly higher than that with glucose. This indicates that raw cornmeal is the most suitable carbon source for the efficient production of tylosin from *Streptomyces fradiae* TM-224-4. This result also shows that *Streptomyces fradiae* TM-224-4, using raw cornmeal as the energy source, has potential in the production of tylosin in an airlift bioreactor. The combined use of the high-yield tylosin producer and the simple air-lift bioreactor may make it possible to have a large-scale production and reduce the production cost of tylosin in *Streptomyces fradiae* TM-224-4 culture. In addition, the use of raw cornmeal may decrease the amount of waste corn-sludge, thus protecting the environment.

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