KOR. JOUR. MICROBIOL. Vol. 123~130(1977)

Some Physiological Properties in Relation to the Growth of the Antibiotics Producing *Streptomyces* spp.

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*Streptomyces albus*와 *Streptomyces globosus*의 몇가지 生長生理的 特性에 關하여

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ABSTRACT

In previous paper, it was reported that antibiotic substance such as tetracycline and streptomycin were produced by *S. albus* subsp. and *S. globosus*.

And increase of mycelial growth of two strains, antibiotic production, and changes of *p*H range are extended to approximately 110-130 hrs in fermenting medium, thereafter they decreased with culture period exception of *p*H range.

Two *Streptomyces* spp. required commonly 4-5% starch as carbon sources and 1.5—2.0% soybean meal as nitrogen sources. However, 0.005—0.01M potassium phosphate dibasic, calcium carbonate (6mg/ml in *S. albus* subsp. and 2 mg/ml in *S. globosus*), 0.01—0.03M, magnesium sulfate and 0.01M ferric chloride showed as optimal concentration for the growth of 2 strains. Mineral compoments such as zinc, manganese, cobalt, sodium and copper at the level of 10^{-4} —10^{-6}M were observed. Especially, zinc ion showed toxicity to the growth of 2 strains at 0.005M. In relation with *p*H, there is a little difference in mycelial growth with cultural initial *p*H.

INTRODUCTION

It has referred that many different antibiotics are secondary metabolic products of microorganisms. During their normal life cycle such organisms will grow in an appropriate culture medium until it has produced the maximum number of cells. The limitation of cell production may be set by the supply of oxygen, by the amount of the carbon and energy sources supplied, or by the other nutritional or environmental factors. Once the culture has stopped growing, it enters the stationary phase. Usually in this stage, secondary metabolites begin to be produced (Weingerg, E.G., 1970). And these are stored up in culture broth by method of fermentation.

However, since these organisms differ in producing amount of antibiotics according to cultural conditions, the best condition for antibiotic production and the best growth condition of these strains

should be investigated.

An antibiotic producing quantities become different according to their sorts such as ammonium nitrate, peptone and amino acids, as nitrogen sources, and it is reported that in carbon sources starch is more effective than other carbohydrates for antibiotics production (Majumdar *et al.*, 1967; Bhadra *et al.*, 1973).

And since most Streptomyces producing antibiotics begin to form the antibiotics from the latter period of logarithmic phase in which their nutrients are almost expended, it is very interesting to study physiological characteristics of these on that condition.

In this experiment, physiological studies, cultural conditions, and relationship between the mycelial growth and antibiotic activity of *S. albus* subsp. and *S. globosus* are attempted to investigate the effect of various carbon and nitrogen sources. And also examination of optimal concentration of other ingredients in fermenting medium will be described.

MATERIALS AND METHODS

1. Test organisms

Two strains of Genus *Streptomyces* were used in all experiments. These were the same strains, which were identified as *Streptomyces albus* subsp. and *S. globosus* by the I.S.P. Methods in previous paper.

2. Fermentation method

Inoculum was grown in submerged culture in a medium containing; Bacto-peptone (Difco), 5.0g; Bacto-yeast extract (Difco). 3.0g to 1,000 ml of distilled water and *p*H was adjusted to 7.0-7.2. This medium was cultured at 28°C for 48 hrs. The final mycelial inoculum was centrifuged at 2,000 rpm and the cake was twice rinsed off with sterile distilled water. It was brought up to its original volume with distilled water and 0.5 ml transfer were made to 100 ml portions of fermenting medium contained in 500 ml flasks. The control medium having the following composition (grams per liter): $CaCO_3$, 3.0; K_2HPO_4, 2.0; $MgSO_4 \cdot 7H_2O$, 1.0; NaCl, 2.0; $CoCl_2 \cdot 6H_2O$, 2.3×10^{-5} was used, and the fermenting medium was added to (2.0%) starch and (1.5%) soybean meal for the experiments (Thornberry, Anderson, O'brien, Wagman, and Perlman's early datas were confirmed by replicate experiments).

3. Analytical method

Mycelium dry weight: The harvest broth was filtered at *p*H 4.0-5.0 by suction on papers. The filtrate was centrifuged at 3,000 rpm and filtered papers were dried at 90±5°C for 1 hr, equilibrated at room temperature, and reweighed, the mycelial weights were calculated in mg of dry mycelia/ml.

Antibiotics activity: Antioiotic activity was determined by the disc-plate method (W.W. Davis and T.R. Stout, 1971) and test organism was only used *Staphylococcus aureus* ATCC 6538p on nutrient agar plates.

RESULTS AND DISCUSSIONS

1. Fermentation changes during the growth of *Streptomyces* spp.

As shown in Figs. 1 and 2, changes of cell weight, *p*H and antibacterial activity were investigated during the growth of *S. globosus* and *S. albus* subsp. and Fig. 2 shows that of *S. globosus*. Inspection of Figs. 1 and 2 shows that the peak of

mycelial growth are extended to approximately 110-130 hrs in fermenting medium. It is speculated that mycelial weight run paralleled to inhibition zone of antibiotic activity until 5 day culture.

Therefore, we may be estimate antibiotic activity as mycelial weight until 5 day incubation. Especially, changes of *p*H range are paralleled to the mycelial weight until maximum growth of two strains. However, they are decreased after 130-150 hr of culture period and increased soon again. This decreasing phenomenon seemed that changes of various organic acids were made after maximum antibiotic production.

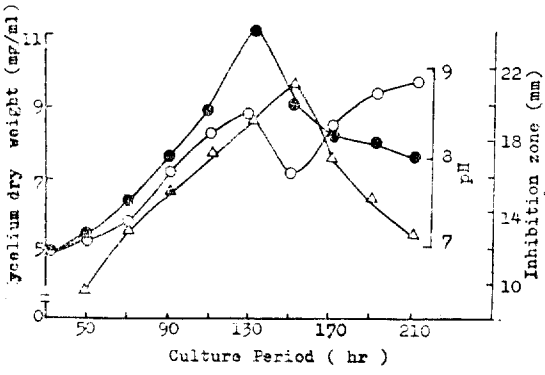

Figure 1. Fermentation changes during the growth of *S. albus* subsp.
○—○; *p*H, ●—●; mycelial wt.,
△—△; inhibition zone diameter.

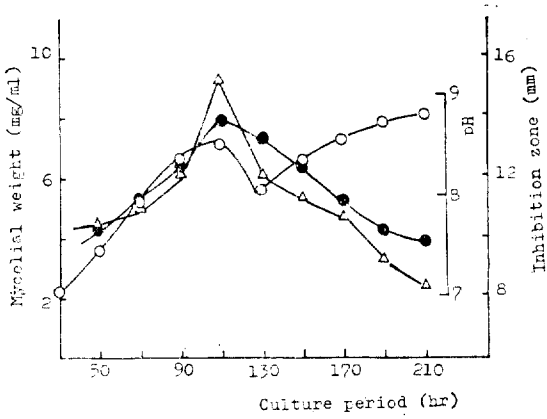

Figure 2. Fermentation changes during the growth of *S. globosus*. ○—○; *p*H ●—●; mycelial wt., △—△; inhibition zone.

2. The effect of the growth on various carbon and nitrogen sources

As early in 1913, Münter described that Streptomyces species could utilize carbohydrates, organic acids and alcohols as carbon sources. Pridham and Gottlieb (1948) tried to characterize Actinomycetes on the basis of carbon compounds.

A number of carbohydrate were investigated for their effect on the growth of *S. albus* subsp. and *S. globosus*. The various carbon sources such as starch, glucose, lactose, and mannitol etc. on the carbon free control media was tested at 3% concentration level. Starch, dextrin and glycerol were excellent carbon sources for their growth and also antibiotic production, maltose, lactose and mannitol were poor carbon sources for their growth, and showed that the weak antibacterial activity. And sodium citrate, inulin, and galactose failed to produce antibacterial substances (Table 1). It was reported that starch was better than glucose for antibiotic production as a carbon source (Majumdar *et al.*, 1967; Bhadra *et al.*, 1973). They explained that this phenomenon is resulted in catabolite repression. On the other hand, in penicillin (Davey *et al.*, 1953), cephalosporin (Darken *et al.*, 1959), and actinomycin (Marshall *et al.*, 1967) have been reported earlier. Fig. 3 shows mycelial production of two *Streptomyces* spp. with various concentration of starch in fermenting medium. In all two strains of *Streptomyces*, 40—50mg/ml of starch concentration was maximum condition of their growth..

The medium for the study of different

Table 1. The effect of various carbohydrate sources on the ahtibiotic production.

	S. globosus		*S. albus* subsp.	
	Mycelial wt. (mg/ml)	Inhibition zone(mm)	Mycelial wt. (mg/ml)	Inhibition zone(mm)
Starch, soluble	8.4	22	10.9	17
Starch, corn	10.2	23	11.4	15
Glucose	6.5	14	8.5	8
Glycerol	9.6	18	11.7	19
Sucrose	6.8	13	9.7	11
Maltose	7.1	18	4.3	—
Lactose	5.9	12	7.0	8
Dextrin	10.3	19	9.9	16
Sodium citrate	3.8	—	3.7	—
Starch, potato	9.2	18	8.6	11
Mannitol	8.3	17	8.8	8
Inulin	7.3	—	8.2	—
Galactose	5.1	—	6.4	—

nitrogen sources was consist of the control medium plus 2.0% starch. The result in Table 2 showed that the highest antibacterial activity and mycelial production of these two strains were obtained in the medium contained soybean meal and beef extract. Glutamic acid was insuitable source for the growth and antibiotic production of them. It was assumed that the inhibition of growth and antibiotic biosynthesis in the presence of many amino-acids or ammonium chloride might be attributed to the acidity of the medium.

The effect on various concentration of soybean meal was illustrated in Fig. 4. Requirement of soybean meal is 15mg/ml and 20 mg/ml concentration.

3. The effect of each ingredients for the growth of *Streptomyces* spp.

To investigate the effect of the miner-

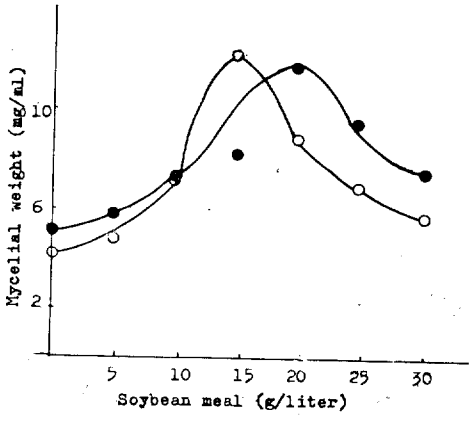

Figure 3. The effect of starch concentration on the growth of *S. globosus*(○—○) and *S. albus* subsp.(●—●)

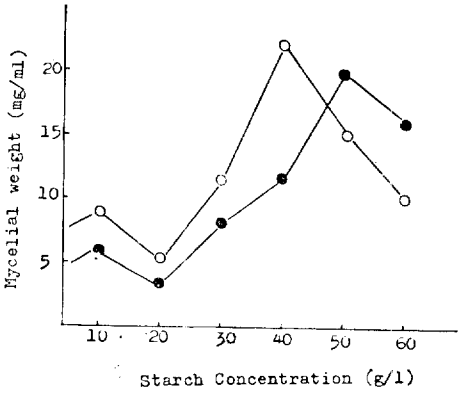

Figure 4. The effect of soybean meal concentration on growth of *S. globosus* (○—○) and *S. albus* subsp.(●—●).

Table 2. The effect of various nitrogen sources on the antibiotic production.

	S. globosus		*S. albus* subsp.	
	Mycelial wt.(mg/ml)	Inhibition zone(mm)	Mycelial wt.(mg/ml)	Inhibition zone(mm)
Beef extract	9.1	19	7.8	17
Peptone	8.7	16	8.9	15
Soybean meal	15.4	24	13.2	21
Peptone(1%)+malt extract(0.5%)	7.9	14	10.8	13
NH_4NO_3	5.7	17	10.8	15
Urea	5.2	—	4.5	—
Glutamic acid	4.7	—	2.9	—

als, one constituent at a time was varied while keeping the others constant in order to confirm requirements and optimized levels of various ingredients. Carbonate is a requirement for growth. The mycelial weight on $CaCO_3$ concentration is shown in Fig. 5, where it can be seen that the concentration of 2 mg/ml and 6 mg/ml is optimal condition for *S. globosus* and *S. albus* subsp. respectively.

Fig. 6 shows the effect of K_2HPO_4.The mycelial weight was effected remarkably on the lower concentration of K_2HPO_4 and the concentration of 0.005-0.01 M phosphate was the minimum level required for maximum mycelium production of two strains. It has been reported that phosphate showed markedly influence on fermentations (Perlman *et al.*, 1952, 1954; Bitti *et al.*, 1954). Our result is agreed with the data of Perlman *et al.* and Bitti *et al.*

The mycelial weight on magnesium sulfate is shown in Fig. 7. Optimum concentration of that is 0.01M and 0.03 M for the growth of *S. albus* subsp, and *S. globosus* respectively.

And the metabolic inhibitors such as

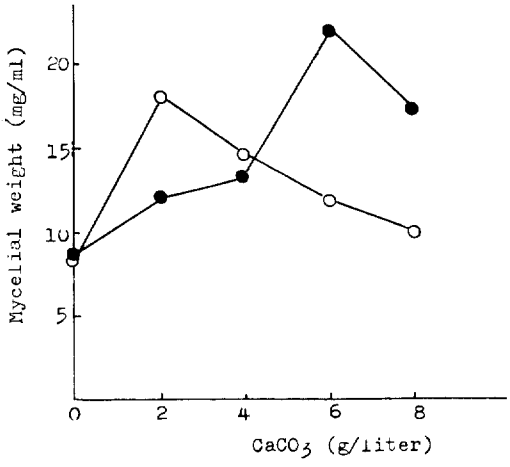

Figure 5. The effect of calcium carbonate on the growth of *S. globosus*(○—○) and *S. albus* subsp.(●—●)

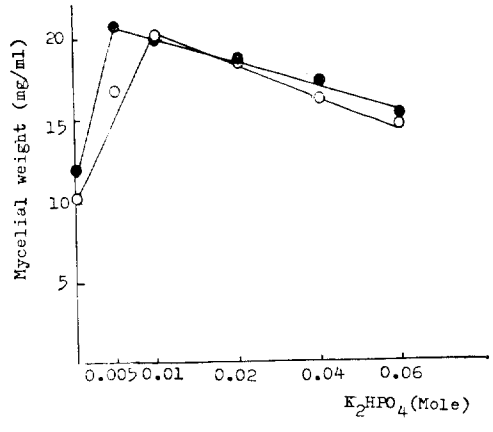

Figure 6. effect of K_2HPO_4 on the growth of *S. globosus*(○—○) and *S. albus* subsp.(●—●).

lysine, cysteine and ferric chloride were added to fermentation media as an attempt to stimulate antibiotic producing activity. Remarkably, ferric chloride was very different from the control. Mycelial weight and antibiotical activity were increased on the basis of the control. Therefore, we attempted to investigate the effect of ferric chloride concentration as shown in Fig. 8. Optimum concentration of ferric chloride is 0.01 M for the growth of *S. albus* subsp. and *S. globosus*.

The requirement trace elements such as sodium, zinc, cobalt and manganese were investigated with the concentration of 10^{-4} to 10^{-6}M. It was found that addition of iron (2×10^{-4}M), manganese (3×10^{-6}M) and cobalt (1×10^{-4}M) increased the mycelial growth of two strains (1.5-2.5 times). None of the other trace element tested was found to be required for the growth. But zinc ion was found to be very toxic to the organisms, and zinc chloride (0.001M) and zinc sulfate (0.0005 M) resulted in decreased cell production as compared with the control.

4. The effect of *p*H and temperature on mycelial weight

In order to study a property of an antibitic substance, it is resulted that *p*H in culture and assay medium as well as in extracting solution and safe keeping solution was very important. The extracting solution may destroy the active drug. That is, many antibiotics sensitive to low or high *p*H may be better extracted at a *p*H near that causing appreciable destruction(Pittillo, R.F., and Wooley, C., 1969).

One of the easiest way to change the sensitivity of an assay system is to change the *p*H. As a general rule, the sensitivity of an assay for antibiotics (penicillins, cephalomycins and monensins) will increase in *p*H, that of basic antibiotics (erythromycin, tylosin and streptomycin) will increase of *p*H (F. Kavanagh, 1970). Antibiotics, *S. albus* subsp. produced, is stable at *p*H 5 and have a high activity. And also antibiotics produced by *S. globosus* is stable at weakly acidic solution.

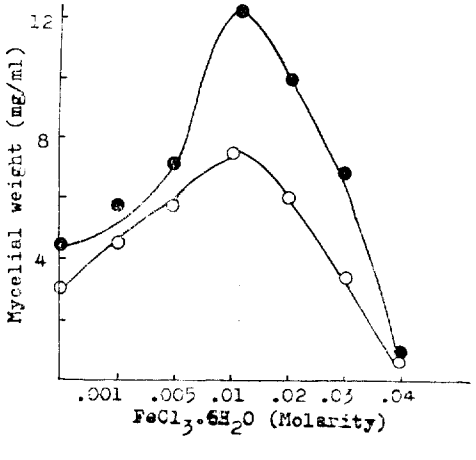

Figure 7. The effect of magnesium sulfate on growth of *S. globosus* (○—○) and *S. albus* subsp. (●—●).

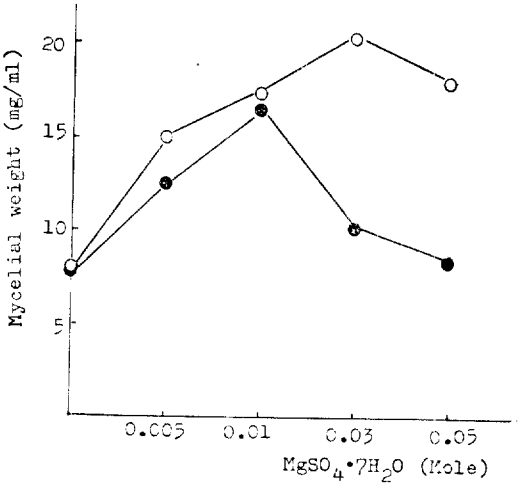

Figure 8. The effect of ferric chloride on growth of *S. globosus* (○—○) and *S. albus* subsp. (●—●).

It is assumed that these facts are related to antibiotics stability with *p*H condition. Dornbush and Abey (1965) reported that all the tetracycline are antibacterially active in weakly acidic than in alkaline media, also that some of the tetracyclines are very unstable when their solution are alkaline.

Growth and mycelial weight were checked with the effect of the initial *p*H which was measured at 5.0, 6.0, 7.0, 7.5 and 8.0 after sterilizing. And the effect on the initial *p*H for the growth of *Streptomyces* spp. during the fermentation process is shown in Fig. 9.

Most of Streptomyces are mesophilic to be at 28°C to 30°C. However, mycelial production is somewhat different by the incubation temperature in submerged shaking culture. In *S. albus* subsp., mycelial production is 12.3mg/ml at 28°C, but 17.3 mg/ml at 38°C, and in *S. globosus*, 11.6 mg/ml both at 28°C and 38°C, similarily.

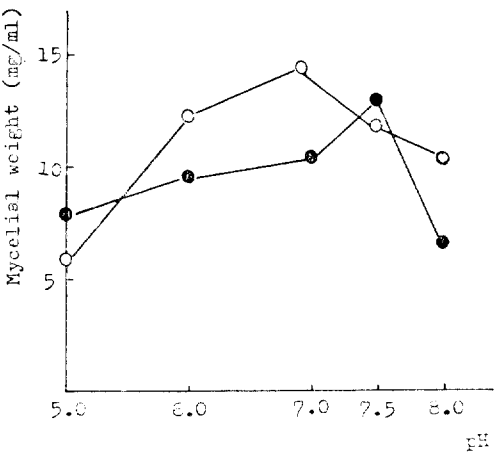

Figure 9. Mycelial production by the initial *p*H(○—○) *S. globosus*, (●—●) *S. albus* subsp.

摘 要

S. albus subsp.와 *S. globosus*에서 Tetracyclin과 Streptomycin 같은 항생물질을 생성된다는 것은 이미 전보에서 밝힌 바 있다.

이들 두 種의 생장최적조건과 항생제 생성정도와의 관계를 배지성분과 그 농도에 따라 검토하였다. 그 결과 *S. albus* subsp.와 *S. globosus* 모두 배양시간이 130~150시간이 될때 항생물질 생성과 균체량이 최고에 달했다. 그리고 *p*H는 그 근처에서만 떨어지는 현상을 볼수 있었다. 또한 두 균주 모두 탄소원으로서는 starch(4~5%) 질소원으로서는 soybean meal(1.5~2.0%)에서 균체의 생장도 양호하고 항균력도 높게 나타났다. 이외의 성분으로는 calcium carbonate(2mg/ml), potassium phosphate' dibasic(0.05M~0.01M), magnesium sulfate(0.01M~0.03M), ferric chloride(0.01M)가 가장 생장에 좋은 배지농도로 추정되며 zinc ion (0.0005M)은 균체생장을 현저하게 억제하였다.

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