한국생물공학회지 제4권 제2호
Korean J. Biotechnol. Bioeng.
Vol. 4 No. 2 162-166 (1989)

Comparative Studies on Streptomycin Producing Strains and Media

Kong Hwan Kim,* Young Ae Joe, Seok Rae Choi and Yang Mo Goo
**Department of Biotechnology, Ajou University, Suwon 440-749*
Department of Pharmacy, Seoul National University, Seoul 151-742

스트렙토마이신 생성균주들과 배지들에 대한 비교연구

*김 공 환, 조 영 애, 최 석 례, 구 양 모
*아주대학교 생물공학과, 서울대학교 약학과

ABSTRACT

When various strains of *Streptomyces griseus* and *S. galbus* were examined for the ability on the production of streptomycin in tryptic soy(TS) broth, S. *griseus* ATCC 27001 was found to be the best. S. *griseus* ATCC 12475 and ATCC 23345 showed also good growth and favorable production of streptomycin. Examination of various complex media reported in fermentation literatures for the industrial production of streptomycin indicated that glucose-soybean meal-sodium chloride (GSS) broth and K (Chucken) broth gave higher yields of streptomycin than others studied by us. Examination of the ingredients of media producing streptomycin in high yield indicated that some components in soybean activated the production of streptomycin. Addition of meat extract enhanced the yield of streptomycin but it could be substituted with distillers' solubles without much effect on the yield. Addition of corn steep liquor decreased the production of streptomycin.

INTRODUCTION

Recently we have performed a series of experiments for the production of aminoglycoside antibiotics. During this work, we found that many industrial strains for the production of streptomycin are currently deposited in American Type Culture Collection (ATCC) and in Institute of Fermentation in Osaka (IFO), and further more that many patents and scientific literatures concerned about the production of streptomycin list many industrial media in which they claimed that the media be very good for the production of streptomycin. Although streptomycin itself has been produced for 30 years of more and its fermentation technology has been well established, we could not find any literature covering systematic studies on the media for the production of streptomycin. Also, we import a large amount of streptomycin every year and we need to establish the fermentation production process in this country. To perform a systematic research on the processes for the production of streptomycin, we could not help clearing out which strains or which meida are really good for the industrial production and for the study of media and the improvement of industrial strains. In this paper, we want to report the results about the production of streptomycin by performing comparative studies of the production of streptomycin by performing comparative studuies of the processes reported by other people

MATERIALS AND METHODS

Microorganisms

Streptomyces griseus ATCC 12475, ATCC 23345, ATCC 15395, ATCC 31087, ATCC e10137, ATCC 27001, IFO 3357, NRRL B-2682 and W. galbus ATCC 14077 were purchased from American Type Culture Collection (ATCC) or Institute of Fermentation in *Osaka,* or were donated by Nothern Regional Research Laboratory (NRRL), Peoria, ILL, USA. The Organisms were stock-

ed on ISP No. 4 agar slants.

Determination of the amount of antibiotic in borth culture.

The amount of antibiotics in broth culture was determined by paper disc agar diffusion assay method. The test organism was *Bacillus subtilis* ATCC 6633. Calibration curves were obtained with authentic streptomycin purchased from Fluka A.G. Five separate assays were carried out. Identification of the antibiotic produced in broth culture was carried out by bioautography.

Dry weight of mycelium

The mycelium in the cultured broth (1.0ml) was harvested in Eppendorf tube and its dry weight was determined. Five separate weights were averaged to give the final weight.

Production of streptomycin by strains of *Streptomyces griseus* and *S. galbus* in tryptic soy(TS) borth.

Spores of various strains of S. griseus and S. galbus were scratched out from an agar plug(1.8cm) in Petri dish cultured with an organism by a loop and they were used to inoculate in TS broth (50 ml). The broth was incubated at 28℃ on a rotary shaker (180 rpm) for 36 hrs and used as a seed culture. The seed culture was transferred to a production media (500 ml, TS broth), which was incubated under the same condition. During the incubation, the cultured broth (5×1 ml) was analyzed for the growth of microorganisms and for the production of antibiotics.

Examination of the production of streptomycin in various complex media.

Spores of *S. griseus* ATCC 27001 obtained from the agar plate (ISP No. 4) were used to inoculate in the S medium(1) (60 ml), which was incubated for 4 days at 27℃ on a rotary shaker. The seed culture (5 ml) was used to inoculate 8 complex media (100 ml). The complex media were cultured under the same condition and examined for the production of streptomycin. The complex media were the Waksman broth (2): glucose 10 g, peptone 5g, meat extract 5g (instead of meat extract, beef extract was used), NaCl 5g, in tap water 1L(pH 7.0, adjusted); GSS (Glucose-Soybean meal-Sodium chloride) broth (3): glucose 25g, soybean meal 40 g, Distilleres' solubles 5g, NaCl 2.5g, in distilled water (1 L, pH 7.0); GSP(Glucose-Starch-Peptone) broth: glucose 15g, starch 5g, peptone 10g, yeast extract 3g, K_2HPO_4 50mg, $(NH_4)_2SO_4$ 2.5g, NaCl 3g, and beef extract 5g, in distilled water (1L, pH 7.0); chuken broth(K broth) (4):glucose 20g, meat extract (instead of meat extract, beef extract was used) 1.0g, soybean flour 10g, KCl 4g, dry yeast (instead of dry yeast, yeast extract was used) 2.5g, $(NH_4)_2SO_4$ 5.0g, $CaCO_3$ 4.0g, K_2HPO_4 0.2g, in distilled water (1 L, pH 7.0, adjusted); GSA(Glucose-Soybean-Ammonium sulfate) broth (5): glucose 60g, soybean meal 30g, corn steep solid (the solid precipitated in corn steep liquor purchased from Sigma was used) 4g, $(NH_4)_2SO_4$ 9g, NaCl 2.5g, KH_2PO_4 0.025g, $CaCO_3$ 0.5g, soybean oil (Dong Bang Yu Ryang Co.) 7.0g, in distilled water (1L); and GA (Glucose-Ammonium sulfate) broth (6): glucose 70g, $(NH_4)_2SO_4$ 60g, lard oil (the oil extracted from the lard fat by heating to about 50℃ was used) 2g, in distilled water (1L, pH 7.0). The glucose in each medium was autoclaved separately.

RESULTS AND DISCUSSION

Many streptomycin producing strains are currently stocked in American Type Culture Collection and in other institutes. When we studied various streptomycin producing strains for the growth and for the production of streptomycin in TSB broth, S. *griseus* ATCC 27001 produced the highest amount of streptomycin (Figure 2). The production of streptomycin and the characteristics of growth of other strains are shown in Figure 1. *S. griseus* ATCC 23345 grew best but the production of streptomycin was low. All the streptmycin producing strains showed characteristic patterns of growth and production of antibiotics; they started to produce antibiotics at the log phase of the growth and showed maximum production at the stationary phase. The pH of the broth was slowly increased to pH 8.5-9.0. *S. galbus* ATCC 14077 and *S. griseus* ATCC 31087 showed poor growth in TS broth. Survey of literatures indicated that Schat et al.(2) used a medium containing glucose, meat extract, peptone, and sodium chloride (Waksman broth) at the early time. The effect of other carbon sources (7,8,9) nitrogen sources (10), supplemented minerals (11) and vitamins (12), and the effect of concentration of the components (13) in the Waksman broth have been studied. Later several other industrial media, such as Glucose-Soybean meal-Sodium chloride (GSS; 3), Glucose-Star-

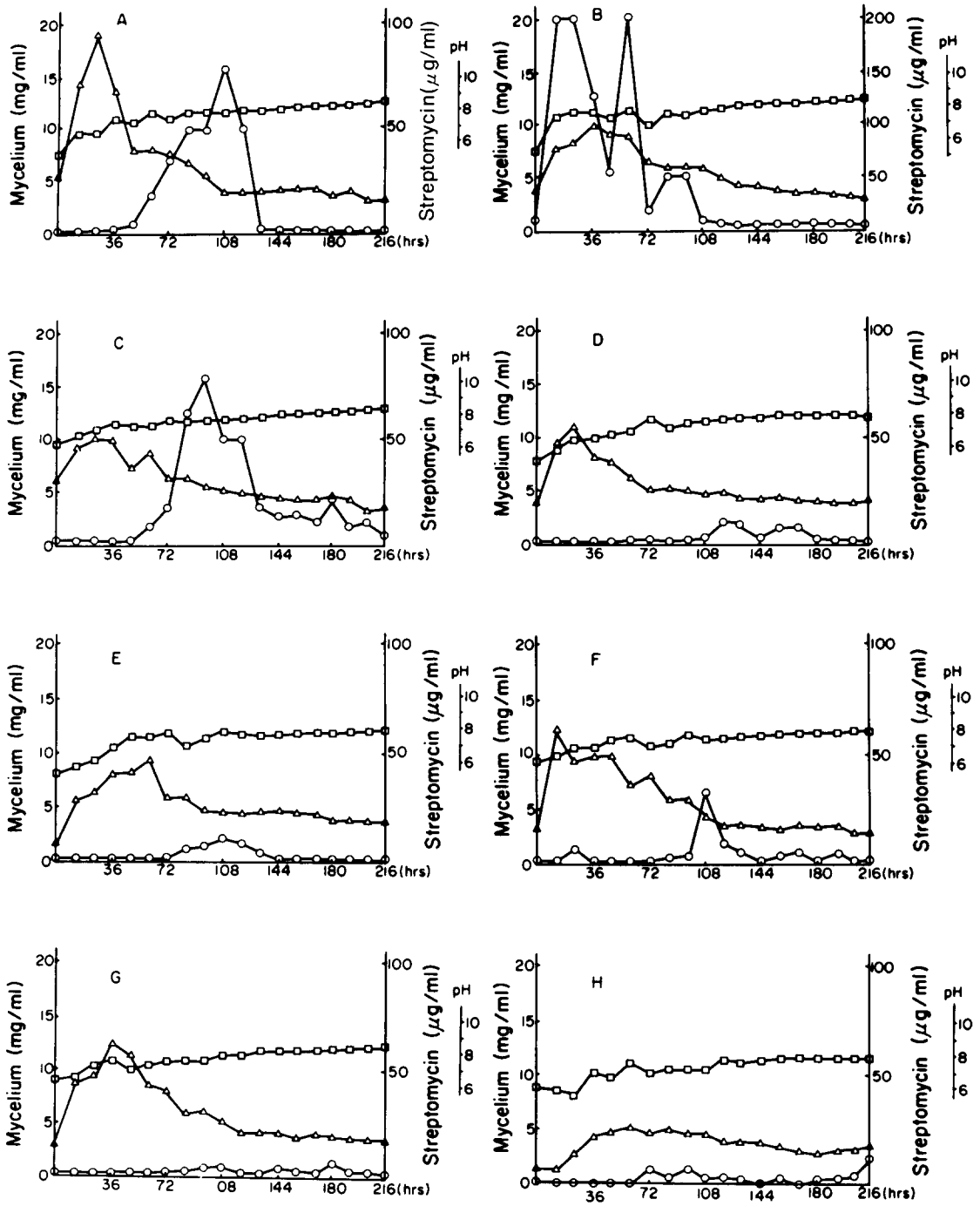

Fig. 1. The growth (△ – △), the amount of streptomycin (○ – ○), and the pH (□ – □) of the broths cultured with streptomycin producing *Streptomyces* in TSB broth. A: *S. griseus* ATCC 23345; B: *S. griseus* ATCC 12475; C: *S. griseus* ATCC e10137; D: *S. griseus* ATCC 15395; E: *S. griseus* ATCC 31087; F: *S. griseus* NRRL B-2682; G: *S. griseus* IFO 3357; and H: *S. galbus* ATCC 14077.

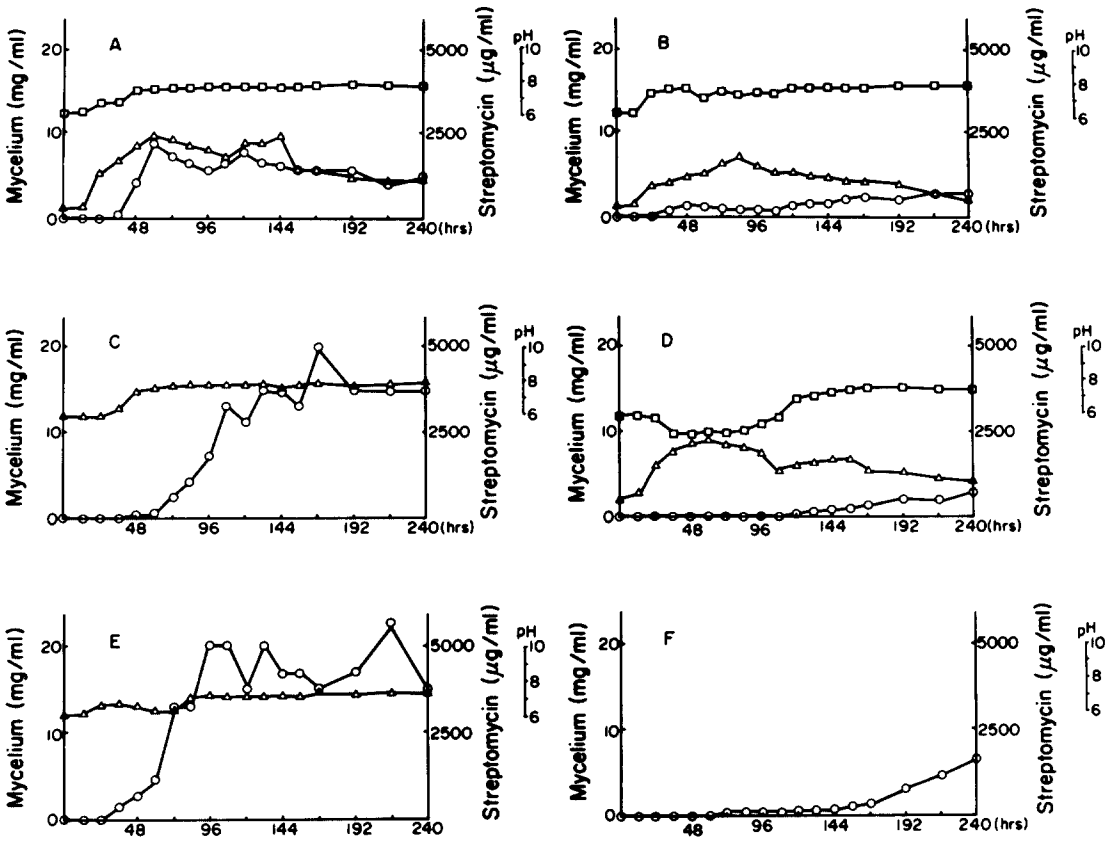

Fig. 2. The growth (△ — △), the amount of streptomycin (○ — ○), and the pH (□ — □) of the broths cultured with *S. griseus* ATCC 27001 in the various complex media. A: TSB medium; B: Waksman medium; C: GSS medium; D: GSP medium; E: Chucken medium; and F: GSA medium.

ch-Peptone (GSP), Chuken broth (K broth; 4), Glucose-Soybean-ammonium sulfate broth (GSA; 5), and Glucose-Ammonium sulfate (GA; 6) were used for the production of streptomycin. We compared these complex media and found that the K broth was the best one among the ones we examined for the production of streptomycin. The results of our comparative study are summarized in Figure 2. The K medium was good in many respects; at the short period of fermentation, high production of antibiotics was observed. However, the media containing meat extract make industrial application difficult. The GSS medium, which is composed of simple ingredients, showed maximum production of antibiotics after 7 days' culture at the same level as that of the K medium. GSA medium and TS broth were also comparatively good for the production of streptomycin. The TS-broth purchased from Difco or Sigma contains tryptone(17g), soytone(3g), dextrose(1.5g), NaCl(5g), and Na_2HPO_4(2.5g). The Waksman and GSP media were good for the growth but poor for the production of antibiotics. The media(K. GSS. GSA, and TS) showing good streptomycin production contain soybean flour or soybean meal. Some components in soybean seem to activate the streptomycin production as reported by Donovick(14). Donovick already mentioned that beef extract was not required for streptomycin production in a medium containing soybean meal; supplementation of beef extract to the soybean meal broth seems to increase the yield of streptomycin a little. Beef extract could be replaced by distiller solubles as in GSS medium.

Sodium chloride should be supplemented to the soybean meal media for high streptomycin production. Donovick also indicated that certain constituents of the beef extract contaminated the purified streptomycin. Defatted soybean flour was reported to decrease the production of streptomycin by Kazanskaya(15). Partial replacement of glucose with soluble starch was reported to decrease streptomycin yield and to accumulate AcOH in the media by Surikova(16). Dulaney(17,18) reported that complete replacement of glucose in basal medium decrease the production of streptomycin. The GA medium showed poor growth. Different results from those reported by perlman and Wagman(19) were obtained in this study. Lipids seem not utilized very effectively by the organism for the production of streptomycin.

In conclusion, among many industrial strains deposited in ATCC and other institutes, *S. griseus* ATCC 27001 was the best producer of streptomycin; *S. griseus* ATCC 23345 and *S. griseus* ATCC 12475 seem to be acceptable. Among the media reported in the literatures, the GSS medium seems the best for industrial production of streptomycin; the K medium showed alos good production of streptomycin.

요 약

스트렙토마이신 생산균주들인 *Streptomyces griseus*와 *S. galbus* strain들은 tryptic soy (TS) broth에 배양하여 항생제 생산을 조사하였을 때 *S. griseus* ATCC 27001이 가장 좋은 균주로 나타났다. 또한 *S. griseus* ATCC 12475와 ATCC 23345에서도 생장과 항생물질의 생산이 뛰어난 것으로 나타났다. 문헌에 보고된 streptomycin 생산배지들을 조사하여 본 결과 glucose-soybean meal-sodium chloride (GSS) broth와 K (Chucken) broth에서, 조사한 다른 배지들에 비해 더 많은 양의 스트렙토마이신이 생성되었다. 이들 배지에 든 성분 중에 콩속에 든 특정한 성분이 스트렙토마이신의 생성을 활성화 시켰다. Meat extract를 soybean meal과 함께 가하여 주면 항생제 생성이 더 증가되었다. distillers' solubles도 meat extract와 유사한 활성이 관찰되나 corn steep liquor를 가하여 주면 항생제 생산이 감소되었다.

ACKNOWLEDGEMENT

The financial support from Korea Science and Engineering Foundation is acknowledged.

REFERENCES

1. R.E. Bennett (1947), *J. Bacteriol.*, **53,** 254.
2. J. Berdy, A. Aszalos, M. Bostian, and K.L. McNitt (1980), *CRC Handbood of Antibiotic Compounds,* Vol. 1, p. 93-95, CRC Press, Bocaraton, Florida.
3. D.J.D. Hockenhull, K.H. Fantes, M. Herbert, and B. Whitehead (1954), *J. Gen. Microbiol.,* **10,** 353-370.
4. O. Hara and T. Beppu (1982), *J. Antibiot.,* **35,** 349-358.
5. A. Singh, E. Bruzelius, and H. Heding (176), *Eur. J. Appl. Mcrobiol.,* **3,** 37-101.
6. Ger. (East) Patent 137, 945(C.A. **92**: 92705j, 1980).
7. S.L. Brinberg (1958), *Antibiotiki,* **3**, 29-33 (C.A. 68:76910w, 1969).
8. S. Shirato, H. Motoyama, and H. Ueda (1962), *Hakko Kyokaish,* **20,** 416-420(C.A. 59: 15919g, 1963).
9. S.L. Brinberg, L.V. Smirnova, and V.P. Pabatsenko (1968), *Antibiotiki,* **13,** 107-112 (C.A. 68:76910w, 1969).
10. U.S. Patent 2,538,942 (C.A. **45:** 2637h, 1951).
11. C.G.C. Chesters and G.N. Rolinson (1951), *J. Gen. Microbiol.,* **5,** 559-565
12. V. Munteanu, G. Ceacoschi, M. Toma, S. Ionnescu, O. Barbu, S. Merlusca, A. Matei, R. Bostan, and M. Magazin (1981), Rom. RO 74, 984(C.A. **97**:4676a, 1981).
13. Patent 650,187 (C.A. **45**: 6808a, 1951).
14. G. Rake and R. Donovick (1946), *J. Bacteriol.,* **52,** 223-226
15. T.B. Kazanskays (1958), *Doklady Akad. Nauk, S.S.S.R.,* **123,** 561-563 (C.A., 53: 5397g, 1959)
16. E.I. Surikova (1960), *Microbiologia,* **29,** 490-494.
17. E.L. Dulaney (1948), *J. Bacteriol.,* **56,** 305-313.
18. E.L. Dulaney (1949), *Mycologia,* **41,** 1-10.

(Received July 12, 1989)