NOTES

EFFECT OF BARBITAL ON THE BIOSYNTHESIS OF STREPTOMYCIN IN *STREPTOMYCES GRISEUS*

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FERGUSON and co-workers described in 1957 that barbital increased the yield of streptomycin (SM) obtained in shake flasks on a near-synthetic medium (FERGUSON *et al.*)[1]. The yields were, however, low at that time and, consequently one of us (HH) investigated the barbital effect in 1966 with an industrial strain producing more than 9,000 μg/ml of SM. We found no effect neither on a complex nor on a chemical defined substrate (H. HEDING 1966 unpublished data). With a semi-industrial strain DTH3 we decided to re-investigate the barbital effect.

The strain was maintained on a soybean, glucose agar on which it sporulated well. As inoculum for the production flasks was used 5 ml of a 48-hour old shake flask culture run at a rotary shaker (250 r.p.m.).

The production flasks and the flasks for propagation of inoculum (50 ml in 500-ml baffled conical flasks) contained media of the following composition, all concentrations in g/liter.

Complex: Glucose 66, soybean flour, full fatted 34, $(NH_4)_2SO_4$ 13, $CaCO_3$ precipitated 10, NaCl 2.5 and $KH_2PO_4 \cdot H_2O$ 0.25 separately sterilized. Two drops of soybean oil was added to control foaming. pH after sterilization was 6.7~7.0.

Chemically defined: Glucose 66, $(NH_4)_2SO_4$ 15, $CaCO_3$ precipitated 10, NaCl 2.5, $KH_2PO_4 \cdot H_2O$ 0.3 separately sterilized, $MgSO_4 \cdot 7H_2O$ 0.2, $MnSO_4 \cdot 7H_2O$ 0.06, $ZnSO_4 \cdot 7H_2O$ 0.01, $FeSO_4 \cdot 7H_2O$ 0.01. Anti-foam agent was not needed on this medium, the pH after sterilization was 6.8~7.0. The flasks with the near synthetic medium were each added 1 ml of an $(NH_4)_2SO_4$ solution containing 0.12 g with 24-hour intervals. Barbital was added to the test flasks as a solution, pH 7.5 giving a concentration in the substrate of 1 g/liter. The fermentations were carried out at 27°C.

The results are given in Table 1. The table shows a significant positive effect of barbital on the complex medium whereas no effect could be demonstrated on the near defined medium. Examinations of the broths showed that the mycelium of the actinomycete only in the complex substrate containing barbital was not disrupted and partially lyzed. In agreement with this, the barbital-containing broth was thick and foaming. It cannot be excluded that extra feeding of these shake flasks with glucose and $(NH_4)_2SO_4$ could have increased the yield further. The optimal barbital concentration and the complex medium were determined in another experiment. The results are given in Table 2.

Our conclusion is that barbital may exhibit a positive and economical feasible effect on the SM fermentation, but the effect is presumably strain dependent. The biochemical reason for the effect of barbital on *S. griseus* has been described in the literature (M. MUSÍLKOVÁ 1959)[2].

Table 1. Effect of barbital (1 g/liter) on the SM fermentation in shake flasks at 168 hours

Variable	pH	Residual glucose (mg/ml)	Total SM yield in flasks (units)	Average yield (units)	Increase(+) or decrease(−) over control (%)
Complex medium (control)	8.0 7.85	2.5 2.65	188,841 187,248	188,044	—
Complex medium + Barbital	8.05 8.05	2.7 2.5	220,485 216,016	218,250	(+) 16.06
Near-synthetic medium	7.1 7.1	7.55 7.9	160,569 157,651	159,110	(−) 15.38
Near-synthetic medium + Barbital	7.05 7.05	7.25 7.85	155,937 163,072	159,504	(−) 15.17

Table 2. Effect of increasing concentrations of barbital on the yield of SM in a complex medium at 168 hours

Barbital (g/liter)	SM (μg/ml)
0.0	4,500
0.8	5,150
1.5	5,800
2.0	5,500
3.0	4,900

References

1) FERGUSON, J.H.; H.T. HUANG & J.W. DAVISSON, Stimulation of streptomycin production by a series of synthetic organic compounds. Appl. Microbiol. 5: 339~343, 1957

2) MUSÍLKOVÁ, M.: The effect of some inhibitors on streptomycin biosynthesis. Folia Microbiol. 4: 76~81, 1959