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NOTES

Conditions Influencing Antimycin Production by a *Streptomyces* Species Grown in Chemically Defined Medium

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Optimal conditions for producing consistent yields of antimycin A in chemically defined medium included: (i) initial *p*H of 6.8 to 7.1; (ii) no iron supplementation; (iii) 2 g of DL-tryptophan per liter; and antibiotic extraction after 7 to 9 days of growth.

The growth of *Streptomyces antibioticus* in chemically defined medium (5) and conditions for antimycin production (4) therein have been described. In an attempt to adapt a high antimycin-yielding strain, AYB-265 (2), to this synthetic medium, conditions were studied whereby consistent results could be obtained. The initial findings are presented in this note.

The culture medium chosen was that of Ramankutty et al. (4) with the following modifications: DL- for L-tryptophan; ferrous sulfate omitted (except where indicated); and trace elements added per liter of medium as 10 ml of a solution containing 1% $ZnSO_4$, 0.8% $MnCl_2$, and 0.1% $CoCl_2$. Glucose was autoclaved separately and added at the time of inoculation. Optimal initial *p*H values were found to be between 6.8 and 7.1. Antimycin production progressively lowered as the *p*H increased above 7.1. Incubation temperature was maintained at 25 C. All water was deionized, doubly distilled, and autoclaved before use.

The liquid medium was inoculated from 6- to 7-day cultures grown on agar slants enriched with beef extract, Tryptose, yeast extract, glucose, and ferrous sulfate. Distilled water (1.5 to 2 ml) was layered over the slants, and white powdery material was scraped with the aid of a sterile wire and pooled in a sterile flask. A uniform inoculum was utilized in all experiments. For example, when a sample of the pooled suspension diluted 1:10 with distilled water gave an absorbance of 0.15 to 0.16 at 600 nm (13-mm round cuvette), 3.5 ml of the undiluted suspension was introduced into 100 ml of 20% sterile glucose. To each 22.5 ml of liquid medium, 2.5 ml of the glucose suspension was added. Triplicate or quadruplicate cultures were grown in tubes (25 by 200 mm), or 100-ml cultures were grown in 250-ml shake flasks placed at an angle of 15° on an oscillating shaker (120 oscillations/min, 3.8-cm stroke). Cultures were harvested at 6 days unless otherwise indicated.

For antimycin isolation, the entire culture was adjusted to *p*H 0.5 to 1 with concentrated HCl. Packed-cell volume was determined by centrifuging for 10 min at 22,000 × *g* in an LRA Lourdes centrifuge. The supernatant fluid and cells were recombined and extracted twice with dichloromethane; pooled extracts were dried on a rotary evaporator. The residue was dissolved in absolute ethanol, applied as a stripe to a silica gel plate (5 by 20 cm), and developed in 3% methanol-$CHCl_3$ against an antimycin A_3 reference. The desired band was located by its R_F and ultraviolet fluorescence, scraped, and eluted with absolute ethanol. The amount of antimycin produced was determined by measuring absorbancy at 320 nm (Cary 15 spectrophotometer); $\epsilon = 4.8 \times 10^6$ cm² per mole (6).

The effect of iron concentration on antimycin production is shown in Fig. 1. Yield was greatest in the cultures containing no added $FeSO_4$ and dropped markedly as the iron concentration was increased; the final *p*H rose greatly with increasing Fe^{2+}. Presumably, the amount of iron introduced with the original inoculum was sufficient to supply the organisms' needs.

As can be seen in Fig. 2, antimycin production

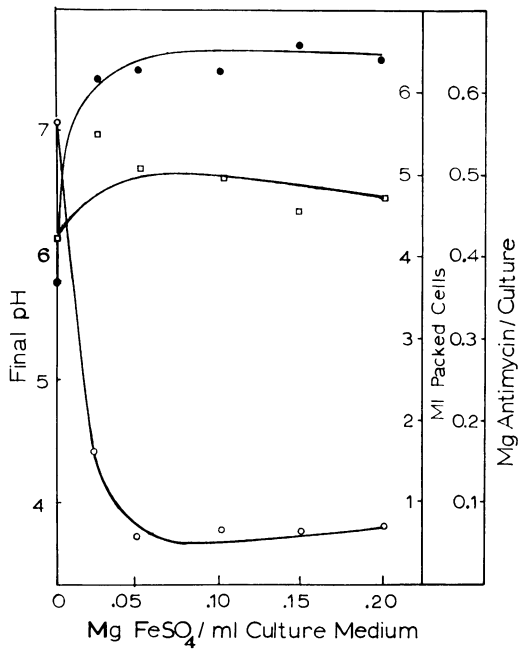

FIG. 1. *Effect of iron on antimycin production. Initial pH = 7.2; 100-ml cultures in 250-ml shake flasks. Symbols: ●, terminal pH; □, packed-cell volume; ○, antimycin (in mg/culture).*

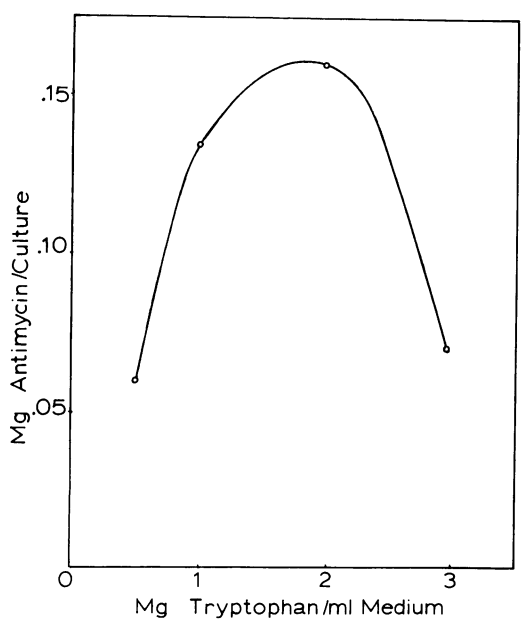

FIG. 2. *Effect of tryptophan on antimycin production. Initial pH was 7.0; 25-ml cultures in 25 by 200 ml tubes.*

increased with the tryptophan concentration up to 2 mg/ml. Higher tryptophan concentrations caused a decreased yield, though cell volume remained constant.

Figure 3 presents a study of packed-cell volume, final *p*H, and antimycin yield as a function of culture age. It was seen that, as the growth phase tapered off, the *p*H of the medium dropped and the amount of antimycin isolated rose markedly. After about 7 days, the *p*H again increased; as it rose to about *p*H 7, the antimycin decreased. It is known that antimycin is unstable at alkaline *p*H (3).

It should be noted that the level of antimycin A produced with the chemically defined medium described in this paper was orders of magnitude lower than that observed by Vezina (7) in a more complex medium (ca. 7 μg/ml versus 1,900 μg/ml). We have found that these yields could be increased to around 10 μg/ml in larger volumes of medium through continuous sparging (Farley, *unpublished data*).

The observation that there appeared to be an optimal DL-tryptophan concentration of ca. 2 mg/ml is of some interest since Neft has found that ^{14}C-benzene ring-labeled DL-tryptophan was

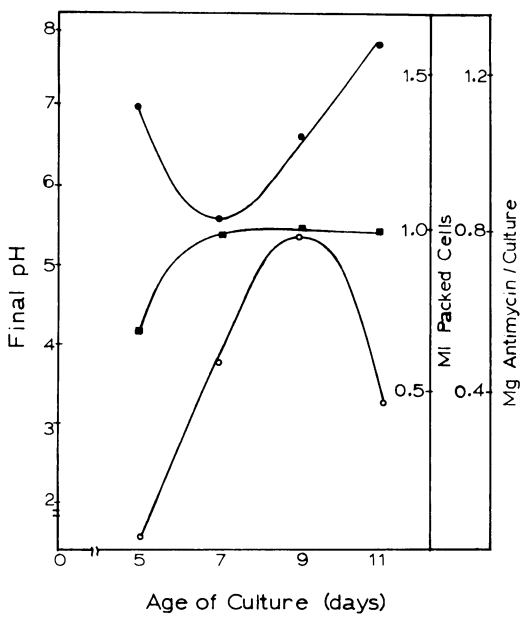

FIG. 3. *Antimycin production, final pH, and packed-cell volume as a function of culture age. The initial pH was 7.1. The medium contained no added iron and was shaken in 25 by 200 mm tubes. Symbols: ●, final pH; ■, packed-cell volume; ○, milligrams of antimycin per culture.*

efficiently incorporated into the aromatic portion of antimycin A (*unpublished data*). Whether the presence of increasing levels of D-tryptophan repressed antimycin synthesis is presently being examined.

Since antimycin A is an amide of a phenolic acid, repression of its synthesis by $FeSO_4$ could be a manifestation of the repressed synthesis of the phenolic acid-synthesizing enzymes. This repression reported in *Aerobacter aerogenes*, *Escherichia coli* (8), and *Bacillus subtilis* (1) occurred in the synthesis of the enzymes involved in the conversion of chorismic acid to 2,3-dihydroxybenzoic acid.

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LITERATURE CITED

1. Downer, D. N., W. B. Davis, and B. R. Byers. 1970. Repression of a phenolic acid-synthesizing enzyme and its relation to ion uptake in *Bacillus subtilis*. J. Bacteriol. **101**:181–187.
2. Kleupfel, D., S. N. Seghal, and C. Vezina. 1970. Antimycin A components. I. Isolation and biological activity. J. Antibiot. (Tokyo) **23**:75–80.
3. Leben, C., and G. W. Keitt. 1948. An antibiotic substance against certain phytopathogens. Phytopathology **38**:899–906.
4. Ramankutty, M., L. V. Kannan, and Z. Rehácek. 1969. Effect of phosphate on biosynthesis of antimycin A and production and utilization of poly-β-hydroxybutyrate by *Streptomyces antibioticus*. Indian J. Biochem. **6**:230–231.
5. Řeháček, Z., M. Ramankutty, and J. Kozová. 1968. Respiratory chain of antimycin A-producing *Streptomyces antibioticus*. Appl. Microbiol. **16**:29–32.
6. Strong, F. M., J. P. Dickie, M. E. Loomans, E. E. vanTemelen, and R. S. Dewey. 1960. The chemistry of antimycin A. IX. Structure of the antimycins. J. Amer. Chem. Soc. **82**:1513–1514.
7. Vezina, C. 1967. Antimycin A, a teleocidal antibiotic. Antimicrob. Ag. Chemother. 1966, p. 757–766.
8. Young, I. G., and F. Gibson. 1969. Regulation of the enzymes involved in the biosynthesis of 2,3-dihydroxy-benzoic acid in *Aerobacter aerogenes* and *E. coli*. Biochim. Biophys. Acta **177**:401.