A number of nutrient media have been described by Goodman (1954), Katagiri (1954), Niedercorn (1952), Petty et al. (1953), Van Dyke and De Somer (1952), and others that allow Streptomyces aureofaciens Duggar to grow in aerated, liquid culture and to accumulate substantial quantities of 7-chlorotetracycline. The concentrations of 7-chlorotetracycline accumulated on these different media vary from about 0.1 to 2.5 g per L. These media are, in general, complex in composition, making it difficult to define the requirements of the organism for growth and to determine the origins of the carbon and nitrogen appearing in the accumulated 7-chlorotetracycline. This paper describes a series of simple, chemically defined nutrient media suitable for growth and 7-chlorotetracycline production by S. aureofaciens strain BC-41, a strain which is characteristic of this species. It is a descendant, through a series of mutation treatments and selections, of the original S. aureofaciens strain A-377 soil isolate of Duggar. The data show that spores of strain BC-41 can germinate and form mycelium, which can produce 7-chlorotetracycline, all in a medium composed of very simple materials.

EXPERIMENTAL METHODS

All fermentations were conducted at 25 C in 250-ml Erlenmeyer flasks containing 25 ml of medium and agitated on a Gump rotary shaker. Washed vegetative inoculum, where used, was prepared by transferring S. aureofaciens strain BC-41 spores to sterile nutrient medium, allowing a 24-hr growth period, centrifuging, washing twice with the original volume of sterile distilled water, and making up to the original volume with sterile distilled water. Four per cent of such inoculum was used to initiate the fermentation, which lasted 5 days. In fermentations inoculated directly with spores (table 3), the spore usage rate was approximately one million spores per ml.

The corn steep liquor-sucrose inoculum medium (Niedercorn, 1952) contains per L: corn steep liquor, 20 g; sucrose, 30 g; (NH₄)₂SO₄, 2.0 g; CaCO₃, 7.0 g. The MB-9A medium is a basal, inorganic salt composition based on corn steep liquor media previously reported by Goodman (1954). It contains per L: CaCO₃, 9.0 g; (NH₄)₂SO₄, 5.0 g; NH₄Cl, 1.5 g; MgCl₂·6H₂O, 2.0 g; KCl, 1.3 g; H₃PO₄, 0.40 g; FeSO₄·7H₂O, 0.060 g; ZnSO₄·7H₂O, 0.10 g; MnSO₄·4H₂O, 0.050 g; CoCl₂·6H₂O, 0.0050 g. This basal mineral salts medium was supplemented with carbohydrate (either starch or sucrose at 55 g/L), amino acids (0.80 g/L of L-histidine and 0.80 g/L of L-methionine), and lipid (either lard oil or glyceryl trioleate at 20 ml/L). The starch was a commercial grade of acid-treated corn starch.

The concentration of 7-chlorotetracycline was determined by fluorometric assay as described by Feldman et al. (1957). The values reported are the averages from fermentations run in triplicate.

RESULTS AND DISCUSSION

The first experiments of this series were carried out in the MB-9A basal mineral salts medium, supplemented with starch and lard oil and inoculated with washed, vegetative inoculum grown on the corn steep liquor-sucrose medium. Under these conditions, about 1.2 to 1.5 g of 7-chlorotetracycline per L was normally produced. A number of substances were added, singly over a range of 0.2 to 2.0 g per L, to this basal medium,
in an effort to increase the concentration of 7-chlorotetracycline produced. Substances showing no significant enhancement of 7-chlorotetracycline production under the conditions tested were: L-alanine, β-alanine, allantoin, α-amino-adipic acid, p-aminobenzoic acid, anthranilic acid, DL-arginine, L-arginine, L-asparagine, L-aspartic acid, butyric acid, L-cystine, dipropionin, gluconic acid, DL-glutamic acid, L(+)glutamine, glutathione, glycine, L-hydroxyproline, L-isoleucine, L-leucine, L-lysine, maleic acid, L-malic acid, malonic acid, DL-mandelic acid, methyl-α-d-glucoside, monacetin, monopropionin, L-phenylalanine, L-proline, salicylamide, L-serine, shikimic acid, sodium succinate, L-sorbosel, L-threonine, L-tryptophan, L-tryosine, urea, and L-valine. Only two substances resulted in reproducible stimulation of the quantity of 7-chlorotetracycline accumulated; these were L-histidine and L-methionine.

Accordingly, in a second series of experiments, graded quantities of both L-histidine and L-methionine were added to the MB-9A-starch-lard oil medium. The results presented in table 1

\[
\begin{array}{|c|c|c|c|c|c|}
\hline
\text{L-Methionine (g/L)} & 0.00 & 0.20 & 0.40 & 0.80 & 1.60 \\
\hline
7-Chlorotetracycline accumulated (g/L) & 0.00 & 1.40 & 1.65 & 1.65 & 1.90 & 1.90 \\
 & 0.20 & 1.95 & 2.55 & 2.60 & 2.45 & 2.60 \\
 & 0.40 & 2.15 & 2.65 & 2.75 & 2.40 & 3.00 \\
 & 0.80 & 2.50 & 3.40 & 3.70 & 4.30 & 3.75 \\
 & 1.60 & 1.75 & 2.20 & 2.30 & 2.60 & 3.10 \\
\hline
\end{array}
\]

* Washed vegetative inoculum grown on corn steep liquor-sucrose medium (see text).
† Starch at 55 g per L; lard oil at 20 ml per L.

Previously reported work with C14-labeled compounds (Miller et al. (1956) ) has shown that some of the above substances (glycine-2-C14, p,L-serine-3-C14, and L-methionine-CH3C14) can contribute carbon to 7-chlorotetracycline synthesized by this strain. The present work is concerned only with net increase in synthesis by added substances, whether by incorporation or by other favorable effects.

\[
\begin{array}{|c|c|c|c|c|c|}
\hline
\text{Glycerol Conc (g/L)} & 0.00 & 0.02 & 0.06 & 0.18 & 0.49 & 0.52 \\
\hline
\text{7-Chlorotetracycline Conc (g/L)} & 0.00 & 0.02 & 0.10 & 0.24 & 0.22 & 0.02 \\
\hline
\end{array}
\]

* MB-9A basal mineral medium, supplemented with glycerol.
show that these two amino acids exert additive effects on the level of 7-chlorotetracycline accumulated. The optimal concentration under these conditions was about 0.80 g of each amino acid per L, and these levels were adopted for further work.

Subsequent experiments took the form of stepwise changes in the direction of more rigorously defined media: (a) washed vegetative inoculum grown on the corn steep liquor-sucrose medium was replaced by washed vegetative inoculum grown on the MB-9A-sucrose-histidine-methionine medium; (b) starch was replaced by sucrose; (c) the amino acids, l-histidine and L-methionine, were omitted; and (d) lard oil was replaced by an equal weight of glyceryl trioleate. The results of these systematic changes are presented in Table 2. Even with all these simplifications, a significant amount (0.85 g/L) of 7-chlorotetracycline was produced. In general, the first three changes reduced the amount of product. Lard oil could, however, be replaced by the purified glyceryl trioleate with equivalent or better results, showing the absence of other essential substances in the crude lard oil.

A further extension of the above work took the form of devising a medium containing only a single, simple organic compound as a source of energy and carbon, all the remaining requirements, including those for nitrogen and sulfur, being satisfied by inorganic salts. Table 3 presents a glycerol-inorganic salt medium allowing mycelium formation from spores and permitting 7-chlorotetracycline biosynthesis.

**SUMMARY**

*Streptomyces aureofaciens* strain BC-41 can germinate from spores, form mycelium, and synthesize 7-chlorotetracycline on a medium containing glycerol as the sole carbon source and ammonium ion as the sole nitrogen source, although, under these conditions, the 7-chlorotetracycline concentrations attained are reduced. The requirements for all the other elements, particularly sulfur, phosphorus, and chlorine, are satisfied by inorganic salts.

**REFERENCES**


