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# INFLUENCE OF PHOSPHORUS ON THE FERMENTATION PRODUCTION OF CHLORTETRACYCLINE (CTC), COBALAMIN (VITAMIN $B_{12}$) AND ANTIFUNGAL ANTIBIOTIC AYF BY STREPTOMYCES AUREOFACIENS

ABOU-ZEID A. ABOU-ZEID and ABD EL-SADEK A. YOUSEF

*Microbiological and Enzyme Chemistry Research Unit, National Research Centre, Dokki, Cairo, U.A.R.*

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An active strain of *Streptomyces aureofaciens* was studied for the fermentation production of chlortetracycline (CTC), an antifungal antiobiotic AYF and cobalamin (vitamin $B_{12}$). The organism could be used for the production of these compounds by regulating the phosphorus concentration in the fermentation medium, low phosphorus concentration was favourable for CTC production, medium phosphorus concentration initiated the organism for the production of AYF, while high phosphorus concentration was suitable for cobalamin biosynthesis. It was also found that phosphorus could control the productivities of the microbial mycelia for the fermentation production of CTC, AYF and cobalamin.

Many investigators pointed to the exceedingly important role of phosphorus compounds in the process of biosynthesis of antibiotics. It was shown that the addition of phosphorus to the fermentation medium decreased the output of streptomycin.[1,2] The same phenomenon was reported in the biosynthesis of chlortetracycline (CTC) by *Streptomyces aureofaciens*.[3,4] The authors clarified the phenomenon by the relationship between CTC and carbohydrate metabolism, which in turn is regulated by phosphorus.

It was found that some strains of *Streptomyces aureofaciens* are capable of producing cobalamin[5–11] (vitamin $B_{12}$).

Kaplan *et al.*[12] isolated an antifungal antibiotic (AYF) from mycelia of *Streptomyces aureofaciens*.

The aim of the present work was to investigate the role of phosphorus in the form of potassium dihydrogen phosphate on the fermentation production of CTC, cobalamin and AYF by an active strain of *Streptomyces aureofaciens*.

## Material and Methods

*Streptomyces aureofaciens* was maintained on medium containing the following ingredients (g/l): glucose 10.0, peptone 5.0, $KH_2PO_4$ 1.0, $MgSO_4.7H_2O$ 0.5 and agar 20.0.

One ml standard inoculum of spore suspension was introduced into 250-ml Erlenmeyer flasks, each containing 50 ml of the following ingredients (g/l): starch 15, corn steep liquor 20 (dry weight). The fermentation medium contained the following compounds (g/l): corn steep liquor (100%) 5.0, $NH_4NO_3$ 5.0, $CaCO_3$ 5.0, NaCl 2.0, starch 30 and $CoCl_2$ 0.0025. The initial pH was adjusted to 7.0 before sterilization. Erlenmeyer flasks of 500 ml capacity, each containing 50 ml liquid were sterilized at 1.2 atmospheric pressure and each flask was inoculated with 1 ml of the vegetative medium containing the experimental organism. The flasks were inserted on a rotary shaker of 200 rev/min at 27–30°C for 72 hr. At the end of the incubation period, the final pH of the fermentation medium and the mycelial dry weight were determined. Perlman's method[13] was used for the determination of CTC. Phosphorus determination was conducted according to the technique of Fiske and Subba Row.[14] The AYF was carried out using the procedure of Kaplan *et al.*[12] Somogyi's method[15–17] was used for the determination of sugar. Vitamin $B_{12}$ was determined spectrophotometrically.[18]

## Results and Discussion

The data of the influence of different concentrations of phosphorus in the form of $KH_2PO_4$ on the fermentation production of CTC, cobalamin and antifungal antibiotic AYF are given in Fig. 1. The results show that the inorganic phosphorus plays an important role in the biosynthesis of these compounds by *Streptomyces aureofaciens*. This is obvious from the results that variable yields of CTC, cobalamin and antifungal antibiotic were obtained.

Concerning the final pH of the fermentation medium, the initial pH of the medium was 7.0 and it was shifted to variable value of pH at the end of the incubation period, with respect to the different concentrations of phosphorus. At low concentration of phosphorus the shift was towards the alkaline side, while at high concentration of phosphorus it was shifted towards neutrality or slight acidic side. This may be due to the presence of high concentration of $KH_2PO_4$. At the same time, the presence of $CaCO_3$ buffered the fermentation medium. Slight variable results of mycelial dry weight were obtained with different concentrations of phosphorus.

The production of CTC was connected directly with the concentrations of phosphorus. High concentrations of phosphorus depressed the yield. The optimal concentration of phosphorus favour-

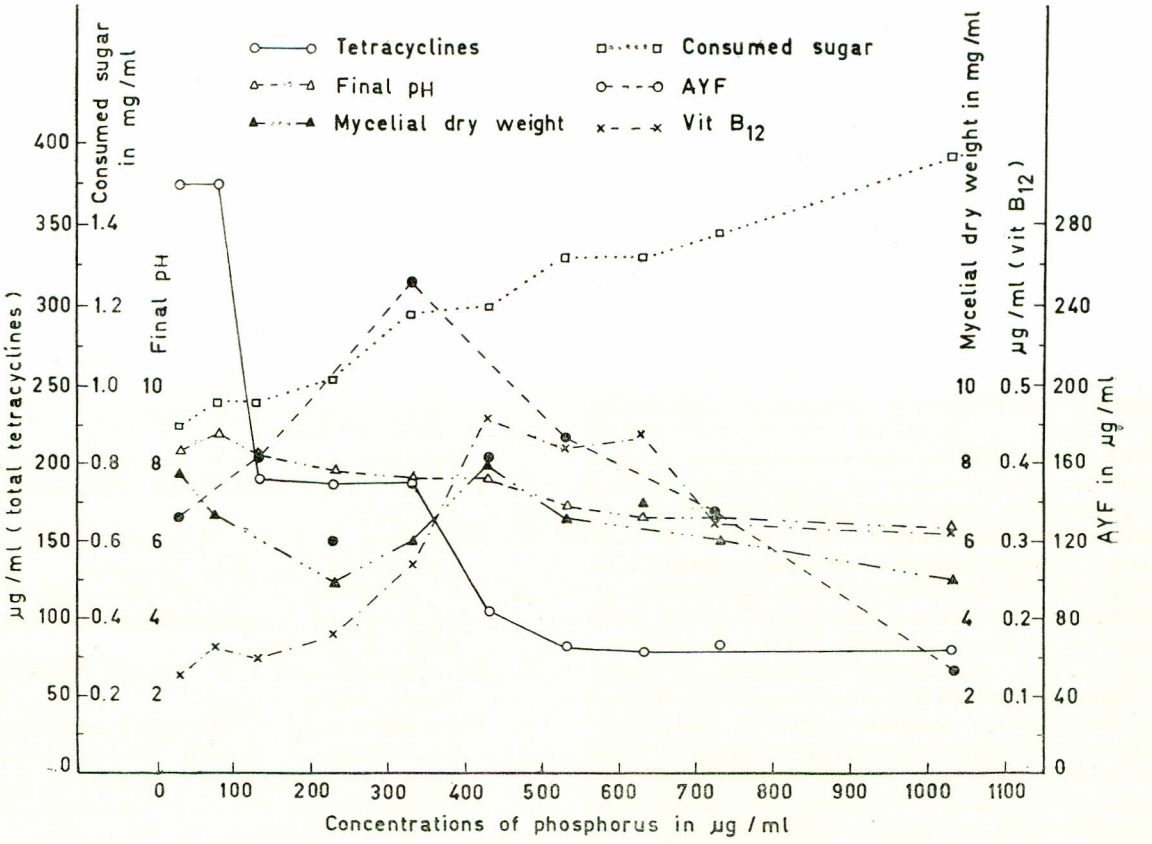
Tetracyclines
Consumed sugar
Final pH
AYF
Mycelial dry weight
Vit B12
µg /ml ( total tetracyclines )
Consumed sugar in mg /ml
Final pH
Mycelial dry weight in mg /ml
µg /ml ( vit B12 )
AYF in µg/ml
Concentrations of phosphorus in µg / ml

Fig. 1

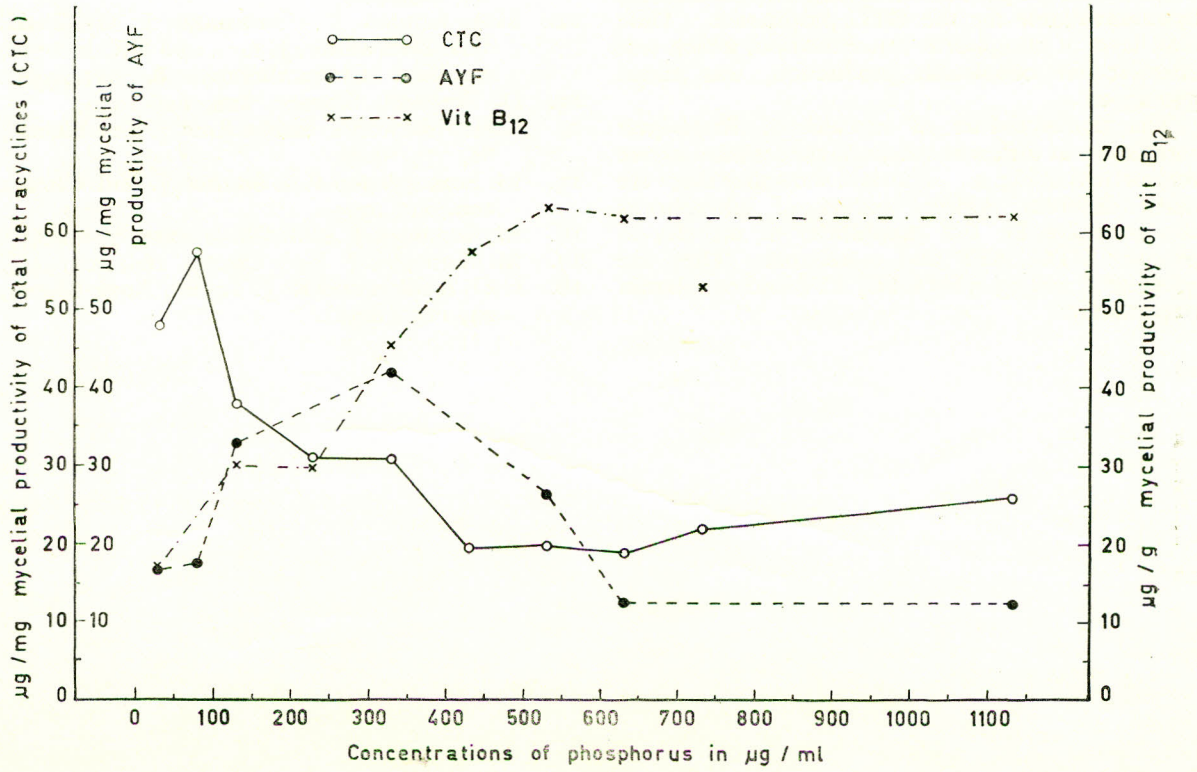
CTC
AYF
Vit B12
µg /mg mycelial productivity of total tetracyclines ( CTC )
µg /mg mycelial productivity of AYF
µg / g mycelial productivity of vit B12
Concentrations of phosphorus in µg / ml

Fig. 2

able for the production of CTC was 80 μg/ml, above which a marked decline in the yield of CTC. was recorded. On the contrary the high concentrations of phosphorus supported the fermentation production of both antifungal antibiotic AYF and cobalamin. In case of AYF, the optimal concentration of phosphorus stimulating the biosynthesis of AYF was 330 μg/ml, while in case of cobalamin it was 630 μg/ml. No sole optimal concentration was obtained to produce valuable yields of the three compounds, but each compound has its optimal concentration.

Variable degree of utilization of starch were obtained by *Streptomyces aureofaciens*. So, in low concentration of phosphorus, the organism utilized starch lower than in high concentration of phosphorus. The presence of phosphorus in high concentration forced the metabolism of the organism to utilize starch. The addition of phosphorus to the fermentation medium inhibited CTC production by shunting a certain amounts of carbohydrate from the hexose-monophosphate pathway into the glycolytic route. It may be supposed that CTC is one of the by-products of carbohydrate metabolism and its accumulation depends on the peculiar conditions met by the microorganism in a particular phase of growth. This shunt of certain amount of carbohydrate may favour the organism to direct its activities for biosynthesising antifungal antibiotic AYF and cobalamin. The carbohydrate in this form was available for the production of AYF and cobalamin, but unavailable for the CTC production. Thus high level of phosphorus concentration, which was effective for cobalamin production, was about 530 μg/ml.

The productivities of mycelia of *Streptomyces aureofaciens* at different values of concentrations are represented in Fig. 2. The data show that there are three different optimal values of phosphorus concentration for the capabilities of mycelia to produce CTC, AYF and cobalamin. They are 57.6, 42.2 and 63.5 for CTC, AYF and cobalamin respectively.

The addition of phosphorus, even in high concentration (1030 μg/ml) did not inhibit the production of cobalamin and also the mycelia of *Streptomyces aureofaciens* was not affected. These results suggest the necessary demand of phosphorus for cobalamin production.

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