Phytochemistry, Vol. 21, No. 5, pp. 1043–1044, 1982.
Printed in Great Britain.

0031-9422/82/051043-02$03.00/0

STIMULATION OF CAROTENOGENESIS IN *BLAKESLEA TRISPORA* BY CUPRIC IONS

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(*Received* 25 *September* 1981)

Key Word Index—*Blakeslea trispora*; Phycomycetes; carotenogenesis; stimulation; trisporic acid; cupric ions.

Abstract—Growth of mated *Blakeslea trispora* in the presence of trace amounts of cupric ions resulted in increases in (a) the amount and rate of initiation of carotenogenesis, (b) the utilization of glucose and Pi and (c) growth. It also caused an increase in trisporic acid synthesis and a two-fold increase in mevalonate kinase activity.

INTRODUCTION

Several physical and chemical agents affect carotenogenesis in a number of systems [1–7]. A variety of compounds have been studied for their effect on carotene synthesis, some important groups being terpenes, ionones, amines, alkaloids and antibiotics. Little is known about the effect of metal ions on carotenogenesis. In the present investigation an attempt has been made to study the effect of cupric ions on carotene synthesis and their possible mode of action.

RESULTS AND DISCUSSION

Metal ions are known to play an important role in secondary metabolism [16]. The effect of the nine biologically important trace metals (atomic numbers 23–30 and 42) [17–19] on the production of the secondary metabolite β-carotene by heterothallic *Blakeslea trispora* was studied. Metal ions at various concentrations were added to the growth medium and inoculated with mated cultures of *B. trispora* and the amounts of carotene measured at the end of 120 hr of incubation. $FeSO_4$ (10^{-7} M) and $MgSO_4$ (10^{-3} M) stimulated carotene production *ca* 1.4- and 1.7-fold respectively, while cupric sulphate (10^{-8} M) proved to be the best, showing some 2.2-fold stimulation of carotene production. Addition of $ZnSO_4$, $CoSO_4$, $MnSO_4$, Na_2SO_4, K_2SO_4 and $CaSO_4$ in the range of 10^{-3}–10^{-10} M did not stimulate carotene production.

It had been observed earlier that the increase in carotene synthesis in this mould in response to penicillin addition is associated with increased utilization of glucose and Pi[10], (Mehta, B. J. and Modi, V. V., unpublished work). In contrast, the stimulation of carotene synthesis induced by mating did not show an increase in the utilization of these nutrients [20]. Consequently, we studied glucose and Pi utilization in the presence of Cu^{2+}. Medium containing 10^{-8} M $CuSO_4$ was inoculated with mated cultures and the utilization of glucose and Pi was studied at various time intervals (Fig. 1a, b). There was an increase in both glucose and Pi utilization in the presence of Cu^{2+}, i.e. the level of utilization achieved by normal cultures in 48 hr was reached by supplemented cultures in 24 hr. This faster utilization of nutrients resulted in maximum growth being achieved much earlier (Fig. 1c); this effect would be expected to lead to an earlier initiation of secondary metabolism. Indeed in normal cultures carotenogenesis was initiated after 48 hr growth, while in supplemented cultures it began at 24 hr (Fig. 1d).

The increase in carotenogenesis on mating is attributed to the production of a sex hormone, trisporic acid, a breakdown product of β-carotene, which derepresses MVA kinase, one of the early enzymes in the carotene biosynthetic pathway [21, 22]. Trisporic acid synthesis in *B. trispora* is known to be catabolically repressed [23]. Studies were conducted on the amount of this synthesized and MVA kinase activity in normal and Cu^{2+} supplemented cultures. Media containing known amounts of added $CuSO_4$ were inoculated with water cultures, incubated for

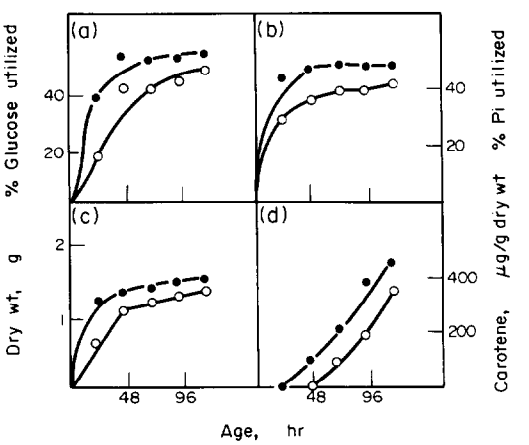

Fig. 1. Glucose utilization (a); Pi utilization (b); growth (c); carotenogenesis (d) in *B. trispora* in the presence (●) and absence (○) of Cu^{2+}.

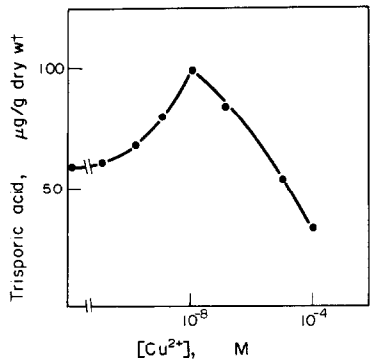

Fig. 2. Effect of various concentrations of Cu^{2+} on trisporic acid synthesis in (±)-*B. trispora*.

120 hr and the amount of acid accumulated was estimated (Fig. 2). There was a two-fold increase in the amount of acid synthesized in the presence of 10^{-8} M $CuSO_4$; higher concentrations were inhibitory.

MVA kinase also showed a two-fold increase in specific activity in the presence of Cu^{2+} after 24 hr growth, going from 1.1 units/mg protein in the absence of added $CuSO_4$ to 2.3 units/mg protein in the presence of 10^{-8} M $CuSO_4$. It is possible, therefore, that Cu^{2+} derepress trisporic acid synthesis by increasing the utilization of glucose and Pi. Increase in the trisporic acid levels brings about increased MVA kinase activity resulting in both earlier initiation of carotene formation and stimulation of carotene synthesis.

EXPERIMENTAL

The plus strain (+) (NRRL 2895) and the minus strain (−) (NRRL 2896) (U.S. Department of Agriculture, Peoria, Illinois) of *B. trispora* were individually maintained as described in ref. [8]. The mould was grown on synthetic Mucor medium (SMM) (pH 6.2) [9]. Inoculum preparation, cultivation and growth measurement was done as described in ref. [10]. Metal ions (as sulphates) were added to the medium at the beginning of each expt. Double glass distilled H_2O was used for the preparation of media.

Trisporic acid was extracted and estimated from the fermentation medium as described by Sutter [11]. All operations were carried out in diffused light between 0° and 4°. The carotenoids were extracted in Me_2CO–Et_2O (1:1) and the total carotenoids were estimated as β-carotene [10]. Glucose was estimated by the arseno–molybdate method [12]. The method of Fiske and Subbarow [13] was used for the determination of Pi. Pi utilization was calculated by subtracting the residual values from the initial values.

The cell free extract was prepared as described earlier [10] and the protein estimated by the method of Lowry *et al.* [14]. MVA kinase (ATP-mevalonate-5-phosphotransferase, EC 2.7.1.36) was assayed by the method of Levy and Popjak [15], using the assay system described by Desai and Modi [10]. A unit of activity is defined as 1 μmol NADPH oxidized/min at 28°.

Acknowledgement—One of us (N.S.G.) acknowledges receipt of a research fellowship from the Department of Atomic Energy, Government of India.

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