mium produces its deleterious effects on the testicular and epididymal functions by inhibiting the ACE and other enzymes and also by disturbing testicular steroidogenesis, sperm production and indirectly thereby epididymal sperm maturation. It is seen that the effect of cadmium on the ACE activity in these tissues does not follow a linear concentration-response relationship The mechanism of the inhibition of ACE by cadmium is under investigation.

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ACCUMULATION OF HISTIDINOL PHOSPHATE IN COPPER TOXIC CULTURES OF *NEUROSPORA CRASSA*

C. SUBRAMANYAM*, G. VENKATESWERLU AND S. L. N. RAO

Department of Biochemistry, Osmania University, Hyderabad 500 007, India.

EARLIER studies on copper toxicity in *Neurospora crassa* from this laboratory have shown that the activity of histidinol phosphate phosphatase (EC 3.1.3.15) was lowered in copper toxicity when the mould was cultured on a medium containing ammonium salts as the sole nitrogen source[1]. Attempts have been made to characterise the accumulated imidazoles in such conditions leading to the identification of histidinol phosphate as an important component of the accumulated imidazoles in copper toxicity. The experimental details and the results are discussed below.

Organism and growth conditions: N. crassa Em 5297 was grown for 72 hr on 10 ml of basal medium containing ammonium tartrate (0.1%) and ammonium nitrate (0.2%) as the nitrogen sources. Copper as $CuSO_4 5H_2O$) (1 mg/10 ml) was included in the medium aseptically to obtain 50% growth inhibited cultures. Similarly manganese as $MnSO_4 H_2O$ (2 mg/10 ml) was included in some flasks to counteract the toxicity of copper and restore the full growth. Growth conditions and harvesting of mycelial pads were as described earlier[1].

Extraction of imidazoles: Mycelial pads were extracted for imidazoles according to Ames *et al*[2]. Three-day old mycelia were washed with cold water and then homogenised with hot water (5 ml/200 mg wet wt of mycelia). The homogenates were kept in boiling water bath for 10 min and the supernatants were collected by centrifugation. The residue was reextracted with 2 ml of hot water and the extracts pooled. The extract thus adjusted to pH 8.5 with saturated $Ba(OH)_2$ and the precipitate formed overnight was discarded by centrifugation. To the clear supernatant, 25% alcoholic $HgCl_2$ (0.02 ml/ml) was added to precipitate the imidazoles. The dried precipitate was dissolved in 0.1 ml of 0.5 N HCl and left overnight. Excess mercury was removed by passing H_2S and the clear supernatant was used for quantitating the histidinol phosphate.

Quantitation of histidinol phosphate by TLC: The extracts obtained as above were subjected to thin layer chromatography on glass plates coated with cellulose in single dimension using *n*-butanol:ethanol:ammonia (sp.gr. 0.88): water (10.1.2.4) and the imidazoles were detected by a diazonium spray reagent[3]. Histidinol, histidine and histidinol phosphate were used as markers and were noted to have an R_f value of 0.6, 0.26 and 0.06 respectively in the solvent system. The area corresponding to histidinol phosphate was scrapped off, eluated with 20% ethanol and again subjected to separation in the same solvent system. Histidinol phosphate in the eluate was quantitated by the diazotisation procedure employing *p*-nitroaniline[4]. The values were corrected for recovery of histidinol phosphate which was also determined with standard histidinol phosphate (recovery was 98.5%).

Table 1 depicts the histidinol phosphate content in extracts obtained from control, copper toxic and manganese supplemented cultures. It is seen that the

TABLE 1

Content of histidinol phosphate in copper toxicity and its reversal by manganese in N. crassa

Type	*Mycelia grown in presence of*		Histidinol phosphate (μg/100 mg wet wt)
	Cu^{2+} (mM)	Mn^{2+} (mM)	
Control	Nil	Nil	145.2 (100)
Toxic	1.57	Nil	216.7 (149)
Reversal	1.57	3.6	146.0 (100)

Figures indicated in parantheses are percentage values. The values are the average of three separate experiments.

accumulation of histidinol phosphate is higher by 49% in copper toxicity and no such accumulation occurred under conditions of reversal of copper toxicity by supplemented manganese.

The present study thus reveals histidinol phosphate as a significant component of the accumulated imidazoles in copper toxicity of the mould. Histidinol phosphate accounted for 30% of the total accumulated imidazoles. While the accumulation of other related imidazoles cannot be ruled out, the accumulation of histidinol phosphate appears to correlate with our earlier observation of a decreased activity of histidinol phosphate phosphatase under conditions of copper toxicity. The reduction in the amount of histidinol phosphate to that of control values, under conditions of manganese reversal, further corroborates our earlier observation that manganese is able to reverse copper toxicity not by limiting the copper uptake but by a specific mechanism, probably by relieving the inhibition of histidinol phosphate phosphatase and promoting the biosynthesis of histidine.

In *Salmonella typhimurium* manganese has been identified as a necessary metal ion for the activity of the bifunctional enzyme imidazole glycerol phosphate dehydrogenase-histidnol phosphate phosphatase and the activity of the enzyme was also shown to be inhibited by copper[6]. The present observation thus supports the view that the mechanism of copper toxicity apparently involves the inhibition of histidine biosynthesis.

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A SIMPLE METHOD TO INDUCE SPORULATION IN *HELMINTHOSPORIUM TURCICUM* INCITANT OF LEAF BLIGHT OF MAIZE

S. SHANKERLINGAM AND
K. A. BALASUBRAMANIAN
Department of Plant Pathology, College of Agriculture, A. P. Agricultural University, Rajendranagar, Hyderabad 500 030, India.

Loss of capacity to sporulate through repeated culturing is one of the difficulties encountered by plant pathologists, while handling axenic cultures of plant pathogens. This poses an acute problem, especially when the pathogen in question is required to be introduced in the form of spores into the suscept.

A common method used to induce sporulation is by exposing the cultures to alternating cycle of 12 hr exposure to near ultraviolet with a radiation peak at 365 nm and extending approximately from 310 nm to 410 nm and 12 hr darkness[1-4]. The incitant of maize leaf blight *Helminthosporium turcicum* sporulates well on the lesions and loses this capacity after a few transfers in axenic culture. A simple method is suggested here to induce sporulation in *H. turcicum*.

The fungus was grown on different media containing extracts of grains of maize, sorghum, barley, wheat and extract of leaves of highly blight susceptible maize cultivar. The final concentration of the extract in the medium was 2%. In addition to the extract the medium contained 2% dextrose also. The media were solidified by using 2% agar agar. Potato Dextrose Agar served as the check.

Two isolates of the fungus (one obtained from Dharwar and the other locally isolated) were used. The isolates were inoculated on media in petri dishes from 6 day old cultures grown on Potato Dextrose Agar. The inoculated petri dishes were incubated at 25° C for 15 days. Each treatment was replicated three times. The sporulation was estimated using a haemocytometer. The data were analysed following a split plot design and are presented in table 1. The highest number of spores were observed on the medium containing maize leaf extract (25.6×10^4 spores/ml)