

Effect of copper and manganese on free amino acid content of Neurospora crassa.

Copper inhibits the growth of a wild-type strain of Neurospora crassa, Em 5297a, and reduces the extractable free amino acid content of the mycelium. Manganese reverses copper growth-inhibition as well as restores mycelial amino acid content.

Neurospora was grown on the medium of Sastry et al. (1962 Biochem. J. 85: 486) at $30^{\circ} \pm 1^{\circ} \text{C}$ with and without 0.1 mg/ml copper ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ = 50% growth inhibitory concentration).

Mycelial dry weights were used to measure the growth. For experiments involving reversal of copper toxicity, manganese was added (0.2 mg/ml $\text{MnSO}_4 \cdot \text{H}_2\text{O}$). Amino acids were extracted from 72 hr. mycelio using 80% ETOH at 80°C (Fuerst and Wagner 1957 Arch. Biochem. Biophys. 70: 31 I). Amino acid analyses were done on 0.2 ml samples of extracts with on automatic amino acid analyser.

TABLE 1

Amino acid $\mu\text{moles/l } 00 \text{ mg}$ dry weight	Wild type mycelia grown on medium containing:		
	Control No additions	100 $\mu\text{g/ml}$ copper	100 $\mu\text{g/ml}$ copper + 200 $\mu\text{g/ml}$ manganese
Aspartic acid	0.9	0.75	0.97
Threonine	1.04	0.62	0.74
Serine	1.12	0.85	1.1
Glutamic acid	4.36	3.86	4.2
Glycine	2.13	1.11	1.65
Alanine	11.4	8.4	9.6
Valine	1.24	1.2	0.81
Methionine	0.21	0.12	0.22
Isoleucine	0.5	0.32	0.32
Leucine	0.48	0.36	0.45
Tyrosine	0.22	co.01	0.21
Phenylalanine	0.16	0.10	0.12
Lysine	1.6	1.53	2.04
Histidine	0.7	0.4	1.1
Arginine	1.99	0.95	1.87
Asparagine + Glutamine	2.0	1.1	1.8
Total ninhydrin positive material ⁺	44.1	29.5	39.3

The lower limit of detection for the analyzer is 0.01 μmoles . Values are average of those obtained in two separate experiments.

⁺Estimated by the ninhydrin method (Rosen 1957, Arch. Biochem. Biophys. 67:10).

Mycelia grown in the presence of copper had lowered levels of thr, ser, glu, gly, ala, met, his, org, gln and asn; tyrosine was totally absent (Table I). Mycelia from manganese-treated copper-inhibited cultures showed almost normal values for each amino acid as well as for ninhydrin positive material. The absence of the tyrosine in copper-inhibited cultures has also been noted by Tandon and Chandra (1962 Naturwissenschaften, 49: 426) in Curvularia penniseti. The growth-inhibitory effect of copper was also reversed by histidine, histidine methyl ester and histidinol but not by histidinol phosphate or by other amino acids.

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