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Electrophoretic variants of L-amino acid oxidase in geographically separated populations of Neurospora

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Abstract

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In Neurospora, the synthesis of the two enzymes, L-amino acid oxidase and tyrosinase, appears to be under a common control mechanism (Horowitz 1965 Biochem. Biophys. Res. Commun. 18:686), possibly involving the synthesis of an unstable common repressor protein (Horowitz et al. 1970 Develop. Biol. 21: 147; 1970 J. Biol. Chem. 245:2784). Both enzymes are induced by starvation, addition of L-ethionine and in cultures undergoing sexual differentiation. Further, the synthesis of both enzymes is affected in the same manner by the protoperithecial-less (ty-1) mutation (Horowitz 1965 Biochem. Biophys. Res. Commun. 18:686). It is not known whether the structural genes for tyrosinase and L-amino acid oxidase are closely linked, as in an operon.

Table 1. Incomplete ordered tetrad analysis of progeny from a cross of ♀ H2000 (LS,A) with ♂ H462 (LF,a). All black and white ascospores in each ascus were isolated, of which many did not germinate.

Incomplete ascus *	Electrophoretic mobility of L-amino acid oxidase R_F Classification **	Mating type	Parental (P) or Recombinant (R)
A1	0.63 LF	a	P
A7	0.56 LS	A	P
B1	0.55 LS	A	P
B2	0.54 LS	A	P
C3	0.54 LS	A	P
C5	0.55 LS	A	P
C6	0.57 LS	A	P
D2	0.60 LF	A	P
D7	0.50 LS	a	R
E3	0.54 LS	A	P
E5	0.56 LS	A	P
F1	0.55 LS	a	R
F6	0.55 LS	A	P

* Each ascus identified by letter. **LS = slow band, LF = fast band.

The structural gene of tyrosinase (T) is known as determined through allelic differences in thermostability and electrophoretic mobility (Horowitz et al. 1960 J. Mol. Biol. 2:96) and is located on the right arm of linkage group I, proximal to al-2, and is at least 30 map units from the centromere. (Horowitz and Fling 1953 Genetics 38:360 and 1956 Proc. Nat. Acad. Sci. U. S. 42:498).

Table 2. Random ascospore analysis of progeny from a cross between H2000 (♀) and H462 (♂).

	Mating type	Electrophoretic mobility	
Parentals	A	LS	9
	a	LF	20
Recombinants	A	LF	30
	a	LS	5
		Total	64

Until the present work, the structural gene of L-amino acid oxidase was unknown and attempts to isolate a mutant were unsuccessful because it plays no essential role in vegetative growth. Consequently, we decided to search for L-amino acid oxidase variants in electrophoretic mobility by comparing this property of the enzyme extracted from a standard laboratory wild-type strain (Emerson) of N. crassa with a Malaysian strain of Neurospora of unknown specific status. These two strains were: H462, a recent ascospore isolated from a cross of Em A with Em a; and H2000, a recent conidial isolate from burnt vegetation in Petaling Jaya, Malaysia.

The electrophoretic mobility of L-amino acid oxidase was determined in polyacrylamide gel electrophoresis (Davis 1964 Ann. N. Y. Acad. Sci. 121: 404) where the substrate was L-histidine HCl. H_2O and the enzyme band was detected by staining with phenazine methosulphate together with MTT tetrazolium. Crude extracts of soluble proteins from 48-hour-old mycelia after further induction by cycloheximide for 45 hours were used. The concentration of cycloheximide required to induce maximal activities of both tyrosinase and L-amino acid oxidase differed markedly in the two strains: H462 and H2000 required the concentrations of 1×10^{-6}M and 1×10^{-8}M, respectively.

The L-amino acid oxidase from both strains appeared as a single band, with that of H2000 having an average R_F of 0.54 (8 determinations) moving more slowly than that of H462 with an average R_F of 0.62 (9 determinations). A mixture of crude extracts of both strains in a proportion of 1:2 (H462:H2000) showed two electrophoretic bands, with R_F values of one corresponding to H462 and another to H2000. This electrophoretic mobility difference segregated as a single allelic difference among progeny from crosses of H462 with H2000, when studied both by tetrad and random ascospore analysis (Table 1 and Table 2). In the tetrad analysis, there was an excess of parentals over recombinants, but the numbers were too few to suggest any linkage between the structural gene of L-amino acid oxidase and the mating type locus in linkage group I. The larger number of random ascospores analyzed showed no or very weak linkage with 29 parentals and 35 recombinants. The possibility of linkage between the structural genes of L-amino acid oxidase and tyrosinase is now being studied.

It is interesting that in these crosses between distantly related strains of Neurospora there seems to be a nucleo-cytoplasmic incompatibility. In the tetrad analysis, out of the 13 viable ascospores from 48 isolated, 10 were of A mating type. These 10 ascospores resembled their female parent (H2000) in carrying the cytoplasm of H2000 and also the mating type chromosome from H2000. Similarly, in the random ascospore analysis, there were 39 ascospores of mating type A out of 64 isolated. These preliminary results indicate that the hybrid ascospore having the cytoplasm of the female parent and the chromosomes of the male may be relatively inviable.

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