Peduzzi, R. and G. Turion. Conidiotion antigen and malate dehydrogenase isoenzyme activities.

We hove recently detected on arc of precipitation produced by on antigenic compound found in the normally conidioting wild type strain Lindegren A of N. crassa but lacking in the morphologic.1 oconidiol mutant amyc (isol.# K422). The arc was found to reappear simultaneously with the recovery of the ability to conidiote upon growth of amyc on acetate and succinate medium (Peduzzi and Turian 1969 Experientia, in press). This antigenic compound is also present in the protein extract of two other normally conidiating strains (Isol.# 15300; isol.# al-Changins, Switzerl.) (Gindrat et al., Mycopathol. Mycol. Appl., in press).

It was therefore interesting to attempt to characterize biochemically this conidial antigen in on effort to understand its eventual physiological role. For that the extract for immunization was prepared by 3 successive freeze (liquid nitrogen)-thawing (30°C) operations followed by lyophilization of the mycelium which was then ground and the resulting Powder extracted with phosphate buffer 0.06 M, pH 7.2, and spun at 10,000 x g in the cold. The method of detection of enzymatic activities on the immunoelectrophoretic patterns according to Uriel (1964 Immunoelectrophoretic onolysis, p. 30. In Grabar and Burtin (ed.), Immunoelectrophoretic onolysis. Elsevier, New York) hos been used to establish the precipitation arcs as enzyme-antibody complexes.

Of the many dehydrogenases which have also been tested, only the malate dehydrogenose (MDH) has been found to be active at the level of the specific arc of precipitation present in the wild type immunoelectrophoretic onolysis (1. E.A.) patterns. However, in addition to this low cathodic mobility MDH-positive arc (MDH_2), two other arcs also show MDH activity in such patterns; these correspond to the MDH isozymes (MDH_1 and MDH3) already recognized with the technique of acrylamide separation in wi ld type N. crassa extracts (Kitto et al. 1967 Arch. Biochem. Biophys. 121:224; Strickland and Shields 1967 Neurospora Newsl. 12: 15). Using the same technique, Loycock et al. (1963 Neurospora Newsl. 4: 20) hove detected a weak addition.1 isozymic bond (MDH_4), as Tsao (1962 Science 136:42) had found using starch gel electrophoresis. On immunochemical grounds, however, it is known that 2 isozymes con react as a single antigen (Pfleiderer et al. 1966 Biochem. Z 346: 269).

By contrast the I.E. A. pattern of the protein extract of the amyc mutant developed with the homologous antiserum to normally oconidiol omyc (on sucrose medium) shows only one positive MDH arc, with low anodic mobility, corresponding to MDH_1 also seen in the wild type pattern. However, when omyc is induced to conidiote on acetate + succinate medium, its 1. E. A. pattern (developed with antiserum of the wild type, containing the conidia specific antibody) shows two MDH positive arcs, not only MDH_1 but also a well defined MDH_2, as recognized by its cathodic mobility and characteristic location.

In conclusion, phenotypic reversion of omyc to conidiotion is accompanied by the appearance of on enzymatically active protein arc. This protein (MDH_2) is induced by acetate simultaneously with the induction of the glyoxylate cycle and the associated processes of gluconeogenesis (Witt et al. 1966 Biochim. Biophys. Acta 128:63). These phenomena ore of particular significance for on understanding of the metabolic orientation required for conidiotion.

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	Isoenzymes MDH		
	1	2	3
wild type	+	+	+
omyc (oconidiol)	+	-	-
amyc (induced to conidiote)	+	+	-