

Conidiation antigen and malate dehydrogenase isoenzyme activities

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Abstract

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and malate dehydrogenase isoenzyme activities. We have recently detected on arc of precipitation produced by an antigenic compound found in the normally conidiating wild type strain Lindegen A of N. crassa but lacking in the morphologic 1 conidial mutant amyc (isol. #K422). The arc was found to reappear simultaneously with the recovery of the ability to conidiate 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 an 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 analysis*, p. 30. In Grabar and Burtin (ed.), *Immunoelectrophoretic analysis*. Elsevier, New York) has been used to establish the precipitation arcs as enzyme-antibody complexes.

Of the many dehydrogenases which have also been tested, only the malate dehydrogenase (MDH) has been found to be active at the level of the specific arc of precipitation present in the wild type immunoelectrophoretic analysis (1. E.A.) patterns. However, in addition to this low cathodic mobility MDH-positive arc (MDH₂), two other arcs also show MDH activity in such patterns; these correspond to the MDH isozymes (MDH₁ and MDH₃) already recognized with the technique of acrylamide separation in wild 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) have detected a weak addition 1 isozymic bond (MDH₄), as Tsao (1962 *Science* 136:42) had found using starch gel electrophoresis. On immunoelectrophoretic patterns, however, it is known that 2 isozymes can 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 conidial omyc (on sucrose medium) shows only one positive MDH arc, with low anodic mobility, corresponding to MDH₁ also seen in the wild type pattern. However, when amyc is induced to conidiate on acetate + succinate medium, its I. E. A. pattern (developed with antiserum of the wild type, containing the conidia specific antibody) shows two MDH positive arcs, not only MDH₁ but also a well defined MDH₂, as recognized by its cathodic mobility and characteristic location.

In conclusion, phenotypic reversion of amyc to conidiation is accompanied by the appearance of an enzymatically active protein arc. This protein (MDH₂) 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 are of particular significance for an understanding of the metabolic orientation required for conidiation. - - - Laboratory of General Microbiology, University of Geneva, Geneva, Switzerland.

	Isoenzymes MDH		
	1	2	3
wild type	+	+	+
<u>omyc</u> (conidial)	+	-	-
<u>amyc</u> (induced to conidiate)	+	+	-