Conidiation antigen and malate dehydrogenase isoenzyme activities

R. Peduzzi
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Abstract
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Peduzzi, R. and G. Turion. Conidiotion antigen 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 Lindegren A of N. crassa but lacking in the morphologic 1 oconidiol mutant omyc (isol. K422). The arc was found to reappear simultaneously of omyc on acetate and succinate medium (Peduzzi and Turion 1969 Experientia, in press). This antigenic compound is also present in the protein extract of two other normally conidiating strains (isol. 15300; isol. 1al-Changins, Switzerland) (Gindrat et al., Mycopathol. Mycol. Appl, in press).

It was therefore interesting to attempt to characterize biochemically this conidial antigen in order 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 immunolectrophoretic patterns according to Uriel (1964 Immunolectrophoretic analysis, p. 30. In Grabar and Burtin (eds.), Immunolectrophoretic 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 mycelium (MDH2), two other arcs also show MDH activity in such patterns; these correspond to the MDH isozymes (MDH1 and MDH2) already recognized with the technique of acrylamide separation in wild type N. crassa extracts (Kim et al. 1967 Arch. Biochem. Biophys. 121:224; Strickland and Shields 1967 Neupora News, 12:13). Using the same technique Loycock et al. (1963 Neupora News, 4:20) have detected a weak addit. 1 isozymic bond (MDH4), as Tao (1962 Science 136:42) had found using starch gel electrophoresis. On immunochemical grounds, however, it is known that 2 isozymes can react as a single antigen (Pfeiferer et al. 1966 Biochem. Z 346: 269).

By contrast the I.E.A. pattern of the protein extract of the omyc mutant developed with the homologous antigen to normally oconidiol 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 omyc is induced to conidiote 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 omyc to conidiolation 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 our understanding of the metabolic orientation required for conidiolation. neg Laboratory of General Microbiology, University of Geneva, Geneva, Switzerland.


Sex hormones (i.e., sex- and fertility-inducing substances) have been isolated from cultures of crosses between Em a and Em a as well as from the homokaryons of Em A and Em a of N. crassa. For the extraction of sex- and fertility-inducing substances from such established cultures, 15 ml of sterilized liquid crossing medium were poured into each Petri dish (size 100 x 15 mm.) in which 5 sheets of sterilized filter paper (1.9 cm) were placed for the support of mycelial growth. Inocula of both Em A and Em a were made simultaneously on the filter paper close to one another and the culture was incubated at a temperature of 23-26°C for three weeks, by which time free ascospores could be detected. A similar procedure was used for single strain cultures (either Em A or Em o), except for the fact that minimal medium was used.

Extraction was made after three weeks of incubation of the cultures. Five grams of activated charcoal (Norit-A) were added to 500 ml of the water extract. The mixture was agitated for a few minutes after which it was kept at room temperature for about 3 hours. Subsequently, as much water as possible was decanted without disturbing the charcoal sediment. The remaining water together with the charcoal was then filtered through filter paper 1 and the charcoal so collected was dried at room temperature. 300 ml of chloroform (reagent grade) were added to the dried charcoal and agitated vigorously for a few minutes, after which the mixture was kept at room temperature for about 3 hours with occasional and mild agitation, after which the chloroform mixture was filtered twice through 5 layers of filter paper 1. This extraction procedure was repeated once more and, finally, about 500 ml of chloroform extract was obtained in this manner. The extract was then evaporated to dryness under vacuum suction. The residue, consisting of a

<table>
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<tr>
<th>Isoenzymes</th>
<th>1</th>
<th>2</th>
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<tbody>
<tr>
<td>wild type</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>omyc (oconidiol)</td>
<td>+</td>
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<td>-</td>
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<tr>
<td>omyc (induced to)</td>
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<td>+</td>
</tr>
<tr>
<td>conidiolation</td>
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The residue, consisting of a