Induction of Conidium Formation in *Neurospora* by Lifting of Catabolite Repression

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INTRODUCTION

In a synthetic, liquid medium with ammonium instead of nitrate as nitrogen source and sucrose (2 %) as the main carbon source, formation of macroconidia on the mycelial mat of *Neurospora crassa* is prevented (Turian, 1964). The anticonidial (repressive) effect of such a medium (M-medium) is reinforced by increasing the sucrose concentration but nullified by lowering it (Oulevey-Matikian & Turian, 1968). The same repressive effect is produced with glucose, and is accompanied by increased ethanol liberation through alcohol dehydrogenase (ADH) reductive activity (Turian, 1972). Acrylamide-gel electrophoresis of mycelial cultures of *Neurospora crassa* grown on the ammonium M-medium revealed only one wide alcohol-dehydrogenase band, but a second presumed to be an ADH with oxidative activity was detected in extracts of conidium-forming, nitrate-grown cultures (DeVecchio & Turian, 1968). A similar oxidative ADH was found by Zink (1969) to be synthesized in *Neurospora* grown on ethanol or in older, sugar-depleted cultures. This suggested a derepression effect which we have studied further in relation to the conidium-inducing effect of our low-sugar ammonium medium. Isocitrate lyase has been included for comparison with the oxidative activity of ADH because of its sensitivity to glucose or catabolite repression (Kornberg, 1959; Flavell & Woodward, 1970; Heick, 1971) and the common link of these enzyme activities with the acetate, conidiogenous metabolism (Turian, 1961; Turian & Bianchi, 1972).

METHODS

Neurospora crassa (wild-type Lindegren A) was grown on modified Westergaard & Mitchell's media (1947), containing either nitrate and 10^{-2} M-potassium citrate to provide a conidiogenous medium, or 10^{-2} M-diammonium citrate replacing the above compounds to provide a myceliogenous medium. The standard 2.0 % (w/v) sucrose content of these media was lowered to 0.2 % (w/v) in half of the ammonium-containing cultures (Turian, 1964).

For studies in enzyme activities, 250 ml Erlenmeyer flasks containing 50 ml liquid media were inoculated with 0.2 ml heavy conidial suspension and incubated for 60 h at 25 °C in partial darkness (short, daily light exposures to stimulate conidiation). The thick vegetative or thinner conidiated mycelia were harvested, washed with distilled water, dried between filter paper and disrupted with quartz sand in cold 0.1 M-tris-HCl buffer at pH 8.5. The homogenates were centrifuged at 10000 g for 20 min at 0 °C and the supernatants used in assays as alcohol-dehydrogenase(s) without any attempt to fractionate this total ADH into its isozymes. For the reductive assay of ADH, the reaction mixture consisted of: 0.2 ml of 0.07 M-acetaldehyde; 0.05 ml of 4×10^{-3} M-NADH; 2.70 ml of 0.1 M-tris-HCl, pH 8.5;

water and enzyme to make 3.0 ml and start the reaction, which was measured by decrease in absorbance at 340 nm during 2 to 3 min (linear reaction rate). For the oxidative assay of ADH, the reaction mixture consisted of: 0.2 ml of ethanol; 0.05 ml of 4×10^{-3} M-NAD; 2.70 ml tris-HCl, pH 8.5; water and enzyme to make 3.0 ml and start the reaction, which was measured by the increase in absorbance at 340 nm during only the first 15 to 30 s because of the high nucleotidase (NADase) activity present in conidial extracts (Zalokar & Cochrane, 1956; Weiss & Turian, 1966). One unit of the enzyme for either forward or reverse reaction was defined as the amount of enzyme causing a change of 0.001 in absorbance per minute, and specific activities were defined as the number of units per milligram of protein in solution. Isocitrate lyase was measured according to Dixon & Kornberg (1959) and its specific activity expressed as $\mu\text{mol glyoxylate/mg protein/h}$. Protein was assayed according to Lowry, Rosebrough, Farr & Randall (1951) and residual sucrose in the filtrates measured with the anthrone reaction (Roe, 1955).

RESULTS AND DISCUSSION

While the standard 2.0 % (w/v) sucrose-ammonium cultures grew for 60 h as only vegetative mycelium, the same cultures with 0.2 % (w/v) sucrose started to form conidia on the surface of their thin mycelial mat after only 48 h growth. These prematurely conidiated cultures harvested at 60 h presented a trend of derepressed enzymatic activities similar to that of 2.0 % (w/v) sucrose – nitrate, normally-conidiated cultures (Table 1).

Sucrose was exhausted in 48 h cultures on low-sucrose medium, but there were still traces in 96 h filtrates of standard ammonium and in 120 h filtrates of nitrate media. For some reason, for example concurrent pentose-phosphate shunt stimulation through NADP-NADPH₂ shuttle (Turian & Bianchi, 1972), nitrate counteracted the repressive effect of 2.0 % (w/v) sucrose, as expressed by the enzyme activities measured as well as by its conidiogenous activity even in the presence of high sucrose concentrations (Hanks & Sussman, 1969). The sugar repression, probably through effects on cyclic AMP or adenyl cyclase, was effective only on the ammonium medium, with high reductive alcohol – dehydrogenase activity as its most evident sign. Such ethanol-producing activity accompanied mycelial growth and reached 7940 units of reductive ADH activity (against 1700 of oxidative ADH activity) after 120 h growth when the sugar was practically exhausted from the medium. The high ratio of reductive to oxidative ADH in assays of the ammonium, high-sugar cultures should correspond to the activity of an essentially fermentative ADH, which was shown to consist of one electrophoretic protein (DelVecchio & Turian, 1968; Zink, 1969). The much lowered ratio of the forward and reverse reactions in the low-sugar cultures suggests the activity of an oxidative ADH corresponding to the second electrophoretic protein from nitrate cultures (DelVecchio and Turian, 1968) and shown by Zink (1969) to consist of at least two isozymes. These oxidative isozymes allow the entry of ethanol into anabolism by oxidizing ethanol to acetaldehyde, as described in yeast (Fowler, Ball & Griffiths, 1972).

That full repression of oxidative ADH activity was not attainable could have been due to the polarization of the contents of hyphae where mitochondria (which may contain part of the oxidative ADH or an independent ADH) are sub-apically positioned, leaving only the ultimate tip as the presumed site of the 'glucose effect' (Turian, 1972). Thus, only the hyphal tip would be the site of a fully effective glucose repression of oxidative enzymes such as a mitochondrial-ADH, with a resulting prevention of mitochondriogenesis, in a similar type of interrelationship to that recently suggested for yeast (Fowler *et al.* 1972).

Our results are in agreement with those of Heick (1971) for *Saccharomyces*, and show

Table 1. *Specific activities of enzymes extracted from 60 h cultures of Neurospora crassa*

Enzymes	Morphogenetic types		
	Mycelial NH ₄ ⁺ : 2.0 % sucrose	Conidial	
		NH ₄ ⁺ : 0.2 % sucrose	NO ₃ ⁻ : 2.0 % sucrose
Alcohol dehydrogenase			
Reductive assay	6610	2210	2400
Oxidative assay	1470	2240	1870
Isocitrate lyase	0.48	1.25	0.99

that the factors which affect the so-called ADH-II (oxidative) activity also affect isocitrate-lyase activity. On the low-sugar medium, the second phase of the diauxic growth is quickly reached and the fungus consumes the ethanol produced in the first phase. Such growth on two-carbon compounds is believed to be possible as a result of a gluconeogenic system involving the glyoxylate cycle induced by the acetate which is generated through ethanol oxidation (Witt, Kronan & Holzer, 1966). Thus, low-sugar conditions mimicked somewhat the simultaneously derepressive and conidiogenous effects of acetate already shown (Turian, 1961), but with agitated liquid cultures which made it impossible to establish a correlation with conidium production (Kobr, Turian & Zimmerman, 1965). Now that we have grown surface cultures on the low-sugar, ammonium medium our view is reinforced that sugar repression versus derepression of enzymes implicated in glycolytic or gluconeogenic processes provides a control mechanism for the morphogenetic alternatives of vegetative extension growth versus conidial differentiation at the hyphal tips.

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