Effect of Glucose, Ammonium and Media Maintenance on the Time of Conidiophore Initiation by Surface Colonies of *Aspergillus nidulans*

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INTRODUCTION

Spore-derived surface colonies of *Aspergillus nidulans* initiate conidiophore and spore development only after a 24 h period of vegetative growth (Axelrod, 1972). In this communication we consider why surface colonies require this 24 h period of vegetative growth before initiating development.

Submerged colonies of *Aspergillus* will initiate development only after they have depleted limiting nutrients which repress development (Galbraith & Smith, 1969). Development of submerged colonies can follow after exhaustion of either carbon or nitrogen sources. We have therefore tested the hypothesis that surface colonies also initiate development only after depleting limiting nutrients. If the period of vegetative growth of surface colonies is due to repression by nutrients then this period would be altered by altering the medium composition; but if repression is not the cause, then the period should be unaffected by changes in medium composition.

METHODS

Organism. A prototroph of *Aspergillus nidulans* strain FGSC4 was used in all experiments.

Media. A defined nitrate-less medium containing 1 % (w/v) glucose as a carbon source, and 10 mM L-glutamic acid (monosodium salt) as a nitrogen source was used as the basic medium. Its composition has been described previously (Gealt & Axelrod, 1974). Where indicated, the glucose concentration was 0.03 % (w/v) instead of 1 % (w/v), and 40 mM-NH₄Cl was substituted for glutamate as a nitrogen source. The media were solidified with 1.5 % agar, or used to saturate absorbent pads supporting sterile membrane filters (Axelrod, Gealt & Pastushok, 1973).

Kinetics of development. Diluted spore suspensions were placed on the surface of agar medium, or membrane filters, incubated at 37 °C, and the resulting number of conidiophores per colony was determined using a $\times 40$ binocular microscope (Axelrod, 1972). The average number of conidiophores per colony was determined for at least 60 colonies from three or more plates. Each experiment was repeated at least three times.

RESULTS

Glucose concentrations of 1 %, and above, and ammonium concentrations of 10 mM and above, are known to repress several enzyme activities in this organism; whereas with glucose concentrations below 0.1 %, and glutamate as nitrogen source, no such repression occurs (Arst & Cove, 1973; Arst & MacDonald 1975; Bartnik, Weglenski & Protrowska,

Table 1. *Effect of carbon and nitrogen sources on conidiophore development*

The time of conidiophore initiation by spore-derived colonies grown on defined medium (A), is taken as the time when there is one conidiophore per colony as shown in Fig. 1. The rate of conidiophore production by colonies grown on defined medium (B), is indicated by the time interval required to double the number of conidiophores per colony after initiation.

Nitrogen source	Carbon source	
	0.03 % glucose	1 % glucose
A. Time of conidiophore initiation		
10 mM-glutamate	23.3, S.D. $\pm$ 0.3 h	24.4, S.D. $\pm$ 1.0 h
40 mM-NH ₄ Cl	23.2, S.D. $\pm$ 0.3 h	24.4, S.D. $\pm$ 1.0 h
B. Rate of conidiophore production		
10 mM-glutamate	54 min	54 min
40 mM-NH ₄ Cl	54 min	30 min

Fig. 1. Kinetics of conidiophore development by spore-derived colonies grown on defined agar medium with 1 % (w/v) glucose as a carbon source and either 10 mM-glutamate (●), or 40 mM-ammonium chloride (▲) as a nitrogen source.

1973; Cohen, 1973; Kinghorn & Pateman, 1974; Roberts, 1963). We have confirmed that 1 % glucose and 40 mM-NH₄Cl are effective in repressing extracellular protease under the conditions in which we measured conidiophore development. However, preliminary experiments revealed that glucose concentrations between 0.01 and 3 % did not affect the period of vegetative growth before surface colonies initiated conidiophore development.

In order to investigate this apparent lack of medium effect, the complete kinetics of conidiophore development were determined for spore-derived colonies grown on either 0.03 % glucose or 1 % glucose as the carbon source, and either 10 mM-glutamate or 40 mM-NH₄Cl as the nitrogen source. The results in Table 1 A show that the time required for spore-derived surface colonies to initiate conidiophore development was not affected by any of these combinations of glucose concentration and nitrogen source. However, the rate of conidiophore production after initiation was affected by certain combinations (Table 1 B). The two parameters of development: (i) time required to initiate conidiophore development, and (ii) subsequent rate of conidiophore development, could be affected independently (Fig. 1).

The observation that changes in medium composition can affect the two parameters of development differently may explain why some investigators have reported large effects of

medium components on the development of certain fungi (Niederpruem & Wessels, 1969; Turian, 1973) and others have reported little or no effects on the same fungi (Schwalb, 1971; Sargent & Kaltenborn, 1972).

In order to determine if depletion of some nutrient other than carbon or nitrogen is necessary to initiate conidiophore development, we maintained initial concentrations of medium components by growing colonies on membrane filters and transferring them hourly to fresh medium (3.6 ml). Colonies grown on filters without transfer initiated conidiophore development at 31.1 , S.D. ± 1.3 h, and colonies transferred to fresh medium initiated conidiophore development at 27.4 , S.D. ± 2.4 h. Since a continuous supply of fresh medium did not prevent initiation of conidiophore development, we conclude that depletion of nutrients was not necessary for surface colonies to initiate conidiophore development.

DISCUSSION

We have shown that the period of vegetative growth of surface colonies preceding initiation of conidiophore development is not altered by either glucose concentrations or nitrogen sources that can affect enzyme activities. In addition depletion of nutrients is not necessary to initiate development of surface colonies. We now come back to the question of why surface colonies require a 24 h period of vegetative growth before initiating development. We have previously reported a gene-controlled activity which is necessary for the acquisition of competence to initiate development (Axelrod *et al.*, 1973; Gealt & Axelrod, 1974). We suggest that the long time period of vegetative growth of surface colonies before initiation of development indicates the time required to complete this endogenous gene-controlled activity, and not that required to deplete exogenous nutrients.

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