SHORT COMMUNICATIONS

Kinetics of Differentiation of Conidiophores and Conidia by Colonies of *Aspergillus nidulans*

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Aspergillus nidulans is a eukaryotic micro-organism that completes its asexual cycle of development in 1 day. Previous studies on the genetics, biochemistry and morphology of this fungus make it a suitable model system for studying differentiation.

Asexual uninucleate conidia can be collected in large quantities and stored under refrigeration. When conidia are placed in liquid growth medium partial synchronous germination is observed (Rosenberger & Kessel, 1967). Increases in DNA, RNA, protein and dry weight precede germ-tube emergence at about 4 h (Bainbridge, 1971). If the medium supports rapid growth, the replication of nuclei in each germ tube is highly synchronized (Kessel & Rosenberger, 1968). During elongation of the hyphal tip there is a wave of nuclear division followed at about 30 min intervals by the formation of a series of incomplete septa (Clutterbuck, 1970). Specific time periods of enzyme synthesis can be detected during exponential growth in batch culture (Carter & Bull, 1969). On solid media the hyphae grow and branch forming a colony the radius of which increases exponentially, for a few hours, and then linearly (Trinci, 1969). The colony continues to increase in radius without differentiation for about 1 day, when it is visible to the naked eye. Then suddenly conidiophores appear, followed a few hours later by the appearance of conidia. At least two gene activities are specifically required in order to convert conidiophores into conidia (Clutterbuck, 1969). In this communication we describe the kinetics of appearance of conidiophores and conidia on colonies of *Aspergillus nidulans* (strain FGSC-4).

The kinetics of appearance of conidiophores on colonies was quantitatively determined as follows: stock suspensions of conidia were diluted in 0.01 % (w/v) Tween 80 (Sigma, St. Louis, Missouri U.S.A.), and 0.1 ml containing about 50 conidia were spread on the surface of media containing 0.5 % Bacto-yeast extract (Difco, Detroit, Michigan, U.S.A.) 2 % D-glucose (Fisher, St. Louis, Missouri, U.S.A.) and 1.5 % Bacto-agar (Difco). After plates were incubated at 36 °C for an appropriate time, colony development was stopped by quickly cooling the plates in a freezer (–15 °C, 10 min) and then storing the plates in a refrigerator until the colonies could be observed microscopically. To count conidiophores the plates were illuminated with a beam of light pointed parallel to the surface of the media, and observed with a ×40 binocular microscope fitted with a net reticule. The surface mycelial growth was hardly visible but individual conidiophores were obvious. Vesicles appeared above the surface as bright smooth spheres on vertical stiff foot cells. For samples taken at each time interval, conidiophores on 15 colonies were counted on each of four plates. The arithmetic mean represents the number of conidiophores per colony.

No conidiophores could be detected before 22 h. At 22.7 ± 1.2 h there was a mean of one conidiophore/colony (Fig. 1). After this time period the number of conidiophores/colony

Fig. 1. Kinetics of appearance of conidiophores and conidia on colonies of *Aspergillus nidulans*. Time after plating conidia on yeast-extract/glucose agar is indicated on the abscissa.

increases exponentially for at least 3 h with a doubling time of approximately 25 to 30 min. Beyond this time the concentration of conidiophores/colony made accurate counts difficult. The time period required to initiate conidiophore development, determined by extrapolation to 1 conidiophore/colony, can be used as a quantitative index of development. Measurements of development in a similar fungus, *Neurospora crassa*, have been devised for mass cultures (Stine & Clark, 1967; Urey, 1971). The techniques we described for measuring development of colonies has the advantage that individual mutant clones, altered in the rate of development, can be recognized and isolated.

The kinetics of appearance of new conidia on colonies was quantitatively determined as follows: conidial suspensions were made by washing plates containing about 50 colonies with 20 sterile glass balls (diameter 3 mm) and 15 ml of 0.01 % (w/v) Tween 80. The concentration of conidia in the suspension was determined by spreading a known volume on yeast-extract/glucose media and counting the colonies formed. This concentration divided by the number of colonies on the plate from which the suspension was made gave the number of conidia per colony. The arithmetic mean of four plates was estimated for each sample. Microscopic examination of such conidial suspensions from 6-day-old plates usually revealed 10^7 single conidia/ml and no hyphal fragments.

The first new conidia appeared $3\frac{1}{2}$ h after the initiation of conidiophore development. At 26.2 ± 0.8 h there was a mean of 1 conidium per colony (Fig. 1). After this time period the number of conidia per colony increased exponentially with a doubling time of about 10 to

15 min. This rapid increase in the number of conidia per colony was a result of the increase in the number both of conidiophores in each colony and of conidia on each conidiophore.

Fungal development can be affected by a variety of environmental factors (Hawker, 1957; Morton, 1961; Galbraith & Smith, 1969). Thus sensitive and quantitative assays for development could be useful to advance morphological observations to the arena of regulatory biochemistry. The time period required to initiate conidiophore development can be considered analogous to the eclipse phase of intracellular phage development. The duration of the developmental eclipse period has been used as an index to determine the developmental response of this fungus to various chemical and physical conditions.

A quantitative measurement of the affect of cycloheximide (actidione) on development was obtained by allowing *Aspergillus nidulans* colonies to differentiate in the presence of this inhibitor. When cycloheximide was present in a yeast-extract/glucose medium, there was a concentration-dependent delay in the time required to initiate conidiophore development, e.g. 1 $\mu\text{g/ml}$ caused a 2 h delay, 30 $\mu\text{g/ml}$ caused a 13 h delay. However, once conidiophore development had been initiated, the rate of increase in number of conidiophores was not altered by the presence of cycloheximide. Thus this inhibitor indicates two stages of development. Cycloheximide may effect development by inhibiting RNA polymerase-1 synthesis of ribosomal RNA (Horgen & Griffen, 1971) required before initiation, or it may inhibit the synthesis of enzymes required for fungal wall production (Katz & Rosenberger, 1971), but not the subsequent function of these macromolecules.

The time period required for *Aspergillus nidulans* to initiate conidiophore development can be used as an index to detect even a small effect on differentiation. Furthermore, since *A. nidulans* grows and differentiates in defined media, the usefulness of this sensitive index of development could be extended by adding test compounds to a minimal salts-sugar medium.

The effect of temperature on the development of *Aspergillus nidulans* was determined by incubating colonies at various temperatures and determining the time period required to initiate conidiophore development. The optimum temperature (minimum time) for wild-type *A. nidulans* to initiate development of conidiophores was about 36 °C. At 31 and 41 °C the developmental eclipse period was about 1.5 times as long as at 36 °C and at 26 °C the eclipse period was about doubled. Thus the technique may be of value for the detection of mutants, the conidiation pattern of which shows an altered temperature response.

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