Viability of Neurospora crassa ascospores after heat activation

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Abstract
Viability of *Neurospora crassa* ascospores after heat activation
Bakerspigel, A. Further observations on the vegetative nuclei of Neurospora crassa. Continued studies with Heily-Newcomer-fixed hyphae of Neurospora crassa have shown (1) that the chromatin of interdivisional nuclei appears as an "interconnected network of strands" (prochromosomes?) containing a number of deeper staining segments. These are visible in Giemsa-stained preparations which have been completely digested with ribonuclease or sufficiently hydrolyzed with hot IN HCl. (2) that the nuclear membrane apparently remains intact until the chromosomes become contracted and more deeply stained. (3) that chromosomes are visible early in division. This is in contrast to Dowding and Weijer's statement that the "chromosomes become most conspicuous in late mitosis" (Dowding, E.S. and Weijer, J. Genetica 32: 339-351, 1962).

A spindle apparatus was not observed when hyphae were fixed with 1% cadmium chloride (Sato, S. Cytologia, 23: 383-394, 1958 and Cytologia 24: 98-106, 1959). Concerning the spindle, Ward and Ciurysek (Amer. Jour. Bot. 49: 393-399, 1962) recently presumed its existence in the somatic nuclei of N. crassa. However, the cytological evidence for a spindle in their photomicrographs is not conclusive. In fact they admitted (p. 395) that "well-defined spindles with distinct fibres were not observed". As far as the writer is aware, additional information or evidence has not been offered by Somers et al. to confirm their earlier observations on the presence, or so-called image, of a spindle (Genetics, 45: 801-810, 1960). Thus, there is as yet no indisputable, cytological evidence for the existence of a classical spindle in the dividing nuclei of N. crassa. ---Department of Bacteriology and Immunology, University of Western Ontario, London, Ontario, Canada.

Brockman, H.E. * Viability of Neurospora crassa ascospores after heat activation. We have been investigating various factors which influence the viability of Neurospora crassa conidia and ascospores in sorbose-supplemented media (de Serres, Kålmark, and Brockman, Nature 193: 556, 1962; and unpublished results). In an experiment designed to test the effect of autoclaving time of the media on ascospore viability, there was an unexpected decrease in viability at 45 min autoclaving (Figure 1). The ascospores were from a cross of a, arg-3, hist-3, nic-2, X A, +, +, ad-3, + and had been heat activated 30-40 min at 60°C in 0.1% agar prior to plating in (A) Fries' basal medium, 0.1% sucrose, 1.0% L-sorbose, 1.5% agar, and 100 µg L-histidine, 100 µg adenine sulphate, and 10 µg nicotinamide/cc, or (B) the same medium without sorbose followed by overplating 9 hr later with the same concentration of sorbose (Newmeyer, Genetics 39: 604, 1954), or (C) the same conditions as (A) except that 0.05% glucose and 0.05% fructose were substituted for the 0.1% sucrose. The media were autoclaved in five lots corresponding to the five autoclaving times, and the ascospores were held, therefore, in 0.1% agar at approximately 24°C for varying periods of time between heat activation and plating.

A second experiment was performed to determine whether the decrease in viability was due to the 45 min autoclaving of the media or to the time of ascospore incubation in 0.1% agar. Ascospores from the same cross were plated immediately after heat activation and at various subsequent times using the same conditions as in (C) of the previous experiment except that 1.5% sorbose and a constant 10 min autoclaving time were used. There was a sharp decrease in ascospore viability at 4 hr and again after 24 hr incubation at approximately 24°C (Figure 2). The reasons for the decrease in viability at 4 hr has not been determined, but it may be due to a heat sensitivity of the ascospores (Lingappa and Sussman, Am. J. Botany 46: 671, 1959) at a particular stage of germination, as the ascospores were plated in 43°C media. This idea is supported by the observation that this decrease does not occur when the ascospores are heat activated and then stored at a temperature (2-4°C) at which germination would not occur (Figure 2).
Figure 1. Effect of autoclaving time on ascospore viability.

Figure 2. Effect of storage at two different temperatures in 0.1% agar between heat activation and plating on ascospore viability.
If these plating conditions are used, it appears necessary either to plate the ascospores immediately after heat activation or to hold them at 0-4°C prior to plating in order to obtain a constant viability.


Davis, R. H., and F. M. Harold. The use of shake cultures of Neurospora for growth experiments.

Many investigators who wish to study certain processes such as the changes of an enzyme activity or the turnover of a polymer during the growth of Neurospora have used replicate stationary cultures. In many such cases, rapidly growing cultures would have been more convenient or meaningful. We have used, in our laboratories, a technique developed by one of us (FMH) which involves fast, nearly logarithmic growth in shaken cultures. Although many similar methods are undoubtedly in current use, it may be useful to describe ours in detail and to discuss some of its advantages.

The shaker used is a New Brunswick Scientific Co. Model R7 "Recipro-Glide" variable-speed reciprocating shaker. The platform may have one or two tiers (the latter may be ordered specially) without flask clamps. Neoprene matting may be used on platforms to prevent movement of flasks, but it is generally unnecessary. For the method described, the shaker is set at a speed of 90-100 cycles per minute, with a 1 1/2-inch stroke. The flasks used are 2500 ml. "low form culture flasks" (Pyrex #4422), five of which will fit on a 26" x 22" platform of the shaker. If 700 to 750 ml. medium is used, the shaker imparts a rotary motion to the medium and provides adequate aeration. Mycelium rarely clings to the flask above the level of the medium unless it is grown well into the stationary phase.

The inoculum for growth is derived from one or more cultures grown on 25 ml. solidified medium in 125 ml. Erlenmeyer flasks. The conidial growth is harvested with sterile water, filtered if necessary, and the suspension is added to the shaker flasks to a final concentration of approximately 10^5 to 10^6 conidia per ml. medium. The flask is capped with aluminum foil rather than cotton, and an air passage is provided by turning up one edge.

In the case of healthy strains, shaken cultures provide 0.75 to 1.5 grams dry weight in 18 hours at 25°C in the various media used. The growth follows an almost logarithmic increase covering three to four doublings in the range of 0.2 to 3.0 grams dry weight per flask. The doubling time is approximately 4.5 hours and growth is complete in about 36 hours. The major period of dry weight increase may be sampled during a 12 to 14 hour period the day after the inoculation of the culture.

Sampling may be done by harvesting aliquots of a single culture (or of a few replicate flasks) at various times during growth without sacrificing the entire culture. As much as half the culture may be withdrawn without altering the pattern of growth. A measured volume of the culture (50 to 750 ml.) is filtered in a Buchner funnel with Whatman No. 1 filter paper, washed, and, if appropriate to subsequent analysis, acetone-dried by pouring acetone over the moist pad. Such a dry pad is convenient in any case for immediate dry weight determination. The dry weights measured in this way are quite reproducible if a large enough conidial inoculum is used to insure a well-dispersed culture. Acetone powders may be made subsequently from the pads by grinding in cold acetone. If the mycelium is to be transferred from one growth medium to another, it may be harvested and washed in a similar manner, but it should not be pressed or drawn to a compact state at any time during the procedure.

The obvious advantages may be stated simply: (1) A kinetic analysis may be performed in relation to many properties of the mycelium, e.g., growth, enzymatic activities, labelling patterns, and response to compounds added before or during growth. (2) With the large inocula and fast logarithmic growth, the mycelium does not contain cells of drastically disparate age. (3) The reproducibility of aliquots allows the use of one to three cultures for a large number of samples. (4) Acetone-dried samples provide a means for monitoring the growth rate of the culture quickly. Some of the applications of this method have been described previously in detail in regard to polyphosphate metabolism (Harold, F. M., 1960, Biochim. Biophys. Acta 45, 172) and in regard to changes in ornithine transcarbamylase activity (Davis, R. H., 1962 Genetics 47, 351). A growth curve is given in the first of the references cited. ---Department of Botany.