Fungal Genetics Reports

Volume 2 Article 17

The use of shake cultures of Neurospora for growth experiments

R. H. Davis

F. M. Harold

Recommended Citation

Davis, R. H., and F.M. Harold (1962) "The use of shake cultures of Neurospora for growth experiments," *Fungal Genetics Reports*: Vol. 2, Article 17. https://doi.org/10.4148/1941-4765.1056

The use of shake cultures of Neurospora for growth experiments

Abstract
The use of shake cultures of Neurospora for growth experiments

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,

University of Michigan, Ann Arbor, Michigan.