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.


Comparison of plate counts with viable counts under the conditions used is necessary. 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,
Horowitz, N. H., and M. Fling. A method for concentrating dilute protein solutions. We have found that dialysis against solid sucrose is an effective way to concentrate dilute solutions of tyrosinase encountered in the course of isolation of the enzyme. The dialysing tubing containing the solution to be concentrated is coiled up in a beaker (or a bucket, if large volumes are involved) and is covered with commercial sucrose. The liquid should be poured off as it accumulates outside the dialysing bag. Dialysis should not proceed for longer than 4 hours, since an excessive concentration of sucrose inside the bag will cause it to burst at the next step. The tubing is removed from the sugar at the end of this time, is tied off above the solution, and is placed in buffer or water to dialyse away the sugar. A 75 to 90% reduction of volume can be obtained in this way, depending on the initial salt concentration in the protein solution. If desired, the entire procedure can be repeated on the concentrate.

Ammonium sulfate can be used instead of sucrose, but we have obtained only a 30 to 50% reduction of volume with its use. In addition, it precipitates, as well as concentrates, the protein.

These methods are superior to the use of polyvinylpyrrolidone or polyethylene glycol (carbowax), in our experience. Both of the latter compounds pass through ordinary dialysing membrane in sufficient amounts to be serious contaminants, and once they get into a protein solution, they are very hard to get rid of. ---Biology Division, California Institute of Technology, Pasadena, California.

Ishikawa, T. A method to improve the fertility of interallelic crosses. To elucidate the genetic fine structure at a locus, the first barrier to be overcome in most cases may be the sterility of the interallelic crosses. In fact, interallelic crosses at the ad-8 locus usually produce a considerable number of perithecia but no ascospores are formed, or when ascospores are formed, almost all ascospores are white or brownish and sterile (fertility, less than 1%). Various attempts have been made to improve the fertility of ad-8 interallelic crosses using variations of crossing media and culture conditions.

A fruitful finding was the fact that lower concentrations of sucrose (0.05 - 0.01%) in the Westergaard and Mitchell's crossing media containing an optimum amount of the supplement (400 μg/ml adenine) was definitely effective in increasing the fertility (up to 30%). With a low concentration of sucrose, the possibility of improving further the fertility has been investigated by supplementing varying amounts of amino acids, purines, pyrimidines, and inorganic salts. None of these supplements seemed to have any effect in improving the fertility. The optimum temperature for interallelic crosses appears to be about 25°C, light has essentially no effect and pH variation shows no significant effect on the fertility within the range 4.0 - 8.0. In place of sucrose, various carbon sources were tested as well. Most carbon sources tested - acetate, citrate, fumarate, lactate, malate, various hexoses, various disaccharides, starch, etc. - were active in supporting crossing as well as growth. Among these substances, acetate gave a higher fertility than other carbon sources (about 40%).

It was noted, however, than a low concentration of carbon also resulted in a reduction in the number of perithecia (less than 10 perithecia in a 20 x 150 mm test tube). In the course of these experiments, it was found by chance that the addition of filter paper as a sole carbon source was effective in producing large numbers of perithecia (more than 20 perithecia in a tube) which shot a considerable number of fertile ascospores. Powdered cellulose and several derivatives of cellulose (e.g., methylcellulose, N,N-diethylaminoethylcellulose, etc.) showed the same effect as filter paper. The addition of a small amount of acetate (0.01 - 0.005%) as another carbon source to the filter paper media seems to give better germination. One third of a circular filter paper (Balstone No. 1; diameter 110 mm) was torn into several pieces and put into the crossing medium in a 125 ml Erlenmeyer flask before autoclaving. A cross made in such a flask gave a significant number of fertile ascospores for an analysis of the fine structure within the ad-8 locus. It may be, however, commented that the fertility problem should be investigated for various possibilities at each locus, since this method was not effective for the ad-4 and ad-5 interallelic crosses.

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