Use of the Caulter counter to measure the numbers and size distribution of macroconidia and microconidia of Neurospora crassa

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Abstract
Measurement of numbers and size distribution of conidia by Coulter counter

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DelVecchio, V. G. and G. Turian. Further studies on the metabolic control of conidiation of *N. crassa*.

Weiss and Turian (1966 J. Gen. Microbiol. 44: 407) demonstrated that conidiogenic cultures (C-cultures) of wild type *N. crassa*, strain Lindegren A, possess lower levels of pyruvate decarboxylase and ethanol dehydrogenase than does the corresponding morphological form.

The mycelial (M)-culture. This prompted the speculation that M-cultures predominate in fermentative enzymes whereas C-cultures have much lower glycolytic activity. The utilization of various inhibitors of this anaerobic mute transformed M- into C-cultures.

In a current morphogenetic study, we noticed that the transformation of C into M could be brought about by the addition of 200 µg of thiamine to 1 liter of C-medium (Turian 1964 Nature 202: 1240). This conversion was accompanied by a three-fold elevation of ethanol dehydrogenase levels and more ethanol detected in the filtrate.

Foster and Goldman (1949 Chemical activities of fungi, p. 316. Academic Press) suggested that the addition of thiamine to cultures of *Rhizopus nigricans*, forced the anaerobic breakdown of glucose and subsequently lessened its aerobic oxidation. This phenomenon centered upon the increased activity of pyruvate carboxylase due to the addition of thiamine. Thus the conversion of C to M by means of thiamine may involve the augmentation of glycolytic enzymes in the normally non-fermentative C-cultures.

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Gillie, O. J. Use of the Coulter counter to measure the numbers and size distribution of macroconidia and microconidia of *Neurospora crassa*.

The Coulter counter (Model A fitted with 70 µ aperture) has been used to measure the size distribution of macroconidia of 74-OR8-la and microconidia from the microconidial fluffy strain Y8743-21 (5-3)A. Figure 1 shows the data obtained plotted as percentage conidio greater than a certain size against relative volume. The volume scale was calibrated using puff ball spores of known size kindly supplied by the Coulter company. On this machine all macroconidia may be counted by setting the sensitivity dial to 4 and the threshold to 10; all microconidia may be measured by setting the sensitivity to 7 and the threshold to 5. If a mixed population of microconidia and macroconidia is present then the relative numbers of each kind may be found by taking readings at the settings described above and subtracting the smaller figure from the larger to obtain the number of microconidia. This computation is quite straight-forward since the sizes of macro- and microconidia do not overlap. The figures obtained for the numbers of microconidia in a given volume measured in this way were in agreement with counts made using a hemocytometer.

It has been found, using this method, that wild type 74-OR8-la produces about 50% microconidia when grown on complete medium slants (without glycerol) at 25°C and few or no microconidia when grown on Vogel's minimal slants at 25°C. The Coulter counter can also be used to measure volume increases of germinating conidia and such volume changes can be observed 1-2 hours before germ tube formation is noticeable. It can also be shown that macroconidia can be differentially separated from microconidia by passive sedimentation in distilled water in a test-tube left to stand over a period of 3 or more hours. McCollan (1957 Contrib. Boyce Thompson Inst. 19: 303) has made a detailed comparison of spore volumes for fungi of many species, including *N. crassa*, using conventional methods of micrometry. Notional Institute for Medical Research, The Ridgeway, Mill Hill, London, N.W.7, England.