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# Efficient method for 32P labeling of conidial DNA

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# Efficient method for 32P labeling of conidial DNA

## Abstract

Efficient method for 32P labeling of conidial DNA

Mandisodza, M. T. and S. K. Dutta. An efficient method for $^{32}P$ labeling of conidial DNA.

One of the major difficulties encountered in attempting to obtain $^{32}P$ labeled DNA from Neurospora conidia is the fact that conidio cannot usually be produced in a liquid medium. The use of a liquid medium is desirable because when conidia are produced on a solid agar medium containing $^{32}P$, the uptake of radioisotope from the medium by the conidio is not efficient enough to give a high specific activity unless very large amounts of 32P are used. A method for growing Neurospora in a liquid medium under conditions that would favor the production of conidio rather than mycelia is what is needed. We have found that a modification of the procedure described by Baker (1969 Neurospora Newsl. 15: 13) gives satisfactory results. Conidia were produced on Fries minimal medium (Ryan, Beadle and Tatum 1943 Amer. J. Botany 30: 784), containing 1/20th the normal concentration of phosphorous and &sorbed in rolls of cotton.

Absorbent cotton sheets were rolled into cylinders of such a size that they formed tightly wedged rings when placed in one-liter wide-mouthed conical flasks (KIMAX No. 26650). Before being placed in the flasks, the cotton cylinders were washed thoroughly with distilled water and squeezed hard to get rid of excess water. After being autoclaved, each flask received the following additions: 50 ml of sterile 8% sucrose solution and 50 ml of sterile 2x Fries minimal medium, containing 1/20th the normal 0.1g% $KH_2PO_4$ and to which 2 x $10^6$ conidia/ml have been added. Approximately 100 ml of water from the washing is retained by the cotton cylinder in each flask. Thus the final concentrations of sucrose, phosphate and conidia were 2g%, 0.05g% and 1 x $10^6$, respectively.

The flasks were incubated at 30°C in the dark for two days and then at ambient temperature in the light for 4-6 days, depending upon the extent of conidiation. Conidia were harvested from the medium by the usual method (Baker ibid.). Specially ground sea sand was used to break open the conidio. Highly purified conidial DNA was isolated using hydroxyapatite chramotography as described previously (Chattopadhyay and Dutta 1969 Neurospora Newsl. 15:11 and Dutta 1969 Neurospora Newsl. 14:9). It was possible to obtain 50 to 100 µg DNA having approximately 10,000 cpm per µg DNA, by using 2 to 3 mc $^{32}P$ (NEN, Boston, Mass) per flask.

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