

Isolation of nuclei from conidia

S. K. Dutta

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Recommended Citation

Dutta, S. K. (1969) "Isolation of nuclei from conidia," *Fungal Genetics Reports*: Vol. 14, Article 11. <https://doi.org/10.4148/1941-4765.2042>

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Abstract

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Dutta, S. K. The isolation of nuclei
from Neurospora crassa conidia.

A greater degree of purity of nuclei isolated from conidial cells of N. crassa is obtained by combining the procedures described by Reich and Tsuda (1961 Biochem. Biophys. Acta 53:574) and Munkres et al. (1966 Neurospora Newsl. 9: 14) for mycelial cells with some modifications made in our laboratory. The

strain used was wild type 74A and all operations are conducted in the cold at 0-4°C. The conidial mass is squeezed dry between Whatman blotting papers and then ground gently with twice its volume of acid-washed sea sand (prepared by powdering the commercially obtained sea sand in a Wiley Mill and passing through a 60-mesh screen) until a smooth paste is obtained. About five volumes of sucrose-EDTA (0.5M sucrose, 1mM NaEDTA, 0.01M Tris HCl, pH 6.5) is added gradually, stirred into a thick paste, and filtered through four layers of silk cloth. The filtrate is centrifuged at 2000 x g for 25 minutes in a refrigerated centrifuge. This crude nuclear pellet is then suspended in a solution containing 0.5 M sucrose, 2 mM EDTA, and 5 mM CaCl₂ at pH 6.5 and centrifuged at low speed (500 x g) for two minutes. Two kinds of pellet were noticed. A hard pellet was formed below the loose pellet. The nuclei contained in the loose pellet were dispersed in their own supernatant and centrifuged again at 500 x g for two minutes. This low-speed centrifugation was repeated until no more hard pellet was noticed.

Comparatively pure nuclear pellet was obtained by passing this final loose pellet through 1.70 M sucrose solution containing 1 mM EDTA. This nuclear pellet was further cleaned from any cytoplasmic attachments by suspending and stirring for two hours in 10 volumes of saline EDTA (0.08 M NaCl, 0.02 M NaEDTA, pH 6.2), sedimenting at 2000 x g and resuspending in fresh solution of saline EDTA. In our process of chromatin isolation from these nuclei, we use triton-X-100 (0.01 %) along with saline EDTA in order to reduce the surface tension of nuclear membrane. The yield of nuclei was low by this process but a consistent purity (as judged by electron microscopy) was obtained, showing a 5:1 (total protein: DNA) ratio. Supported by NSF Grant No. Gy3894. ■ ■ ■ Department of Botany, Howard University, Washington, D. C. 20001.