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Quick and efficient protoplast isolation from Neurospora crassa.

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Abstract

Quick and efficient protoplast isolation from *Neurospora crassa.*

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Protoplasts, as genetic tools, have drawn much attention recently, due to their role in both fusion- and transformation-systems. Spheroplasts are generally produced from germinated conidia; however, they are very heterogeneous in size, physiology and partly surrounded by conidial wall remnants. In order to obtain protoplasts from N. crassa we have used vegetative mycelium of a cell-wall defective mutant, rugged.

According to Brody and Tatum (Proc. Natl. Acad. Sci. USA (1967) 58: 923-930) the phosphoglucomutase enzyme activity of ragged appears to be 6-8% of that of wild type, resulting in a 50% decrease of the β-1,3 glucan content in the cell-walls. Growth of ragged mutants are characterized by frequent hyphal-branching. This indicates that the surface of the growing tips, where the wall synthesis is the most intensive and the wall is most sensitive to the effect of wall-decomposing enzymes, is relatively large. The tip is covered with a wall layer consisting of chitin and protein (Trinci, 1978, Sci. Prog. Oxf. 65: 75-99). Since the ragged strains are defective in conidiation, we prepared hyphal-suspensions from 48 h cultures grown in 2x100 ml of Vogel's liquid medium. After harvesting by centrifugation (3500 g. 4°C, for 10-15 min), mycelia were fragmented in 1 M sorbitol at high speed (15,000 rpm for 15-20 sec) in a Waring blender. The resulting suspensions ($2\text{-}5\times10^7$ fragments/ml) were stored at -70°C in small portions and used to inoculate cellophane-agar media.

Cellophane-agar (Ferenczy et al. 1970 in, Wirkungsmechanismen von Fungiziden, Antibiotika und Cytostatica, Eds. Lyr and Rawald, Akademie Verlag, Berlin, p. 191) was prepared as follows: sterile discs of cellophane (cut as large as the agar surface) were laid on the top of solid Vogel's medium in Petri dishes. Then 50-100 µl of hyphal-suspension was mixed with 200-300 µl of 0.15 M NaCl and spread on a cellophane-sheet. Cultures were grown at 27°C for various periods of time, then used for producing protoplasts. Cellophane cultures were put with their surface down into 4-5 ml of the enzyme solution consisting of a 1% Helicase (Reactifs IBF), 0.1% Chitinase (from Serratia marcescens, Serva), 10 mM MOPS (4-morpholinopropane sulfonic acid), pH 5.8, 1 M sorbitol. Digestion was carried out at 27-29°C, with gentle shaking (100 rpm).

The time of the appearance and the number of protoplasts depend on the age of the culture. If relatively young, i.e. 30-40 h old, many protoplasts can be observed after 10 min of digestion. From a 40-42 h culture (0.5 g of wet weight) about 100-150 million of stable protoplasts are liberated during l-2 h. In our experiments the mycelium is converted to protoplasts with an efficiency of 95-100%.

If the culture is older (about 50-60 hr) the appearance of protoplasts takes a longer time. In this case the number of protoplasts released from 0.5 g of wet weight mycelium is significantly lower (about $2\text{-}5\times10^6$ after 4-5 h).

Protoplasts can be separated from the (occasional) mycelial residue by filtration through glass wool. The size of the protoplasts is almost uniform (about 10 µm in diameter), and they contain, on the average, ten nuclei. As proved by electron-microscopy (V.Zs.-Nagy, VILEG, Hungarian Section University Medical School, Debrecen, Hungary), protoplasts lack cell wall. If 60 mM phosphate buffer is used instead of 10 mM MOPS in the stabilizer solution, the protoplasts are less stable. The protoplast-forming activity of the forementioned enzymes is significantly lower with 0.7 M KC1 than with sorbitol.

If large quantities of protoplasts are needed, 6-8 cellophane-cultures can be placed on one another (separated with discs of sieve-cloth). By this method 25-40 ml enzyme liberates about 10^9 protoplasts. The ragged mutation can be introduced easily to any other N.crassa strain by crossing. (Supported by the Hungarian Academy of Sciences.) · · · Institute of Biology, University Medical School, H-4012 Debrecen, Hungary.