Mitotic Synchrony in Multinucleate
Schizophyllum Protoplasts

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Summary
Protoplasts liberated from the mycelium of Schizophyllum commune were cultivated in an osmotically stabilized medium in the presence of cell-wall degrading enzymes to prevent wall regeneration. Anucleate protoplasts lysed more rapidly than nucleate protoplasts. Growth of nucleate protoplasts was accompanied by repeated synchronous nuclear divisions, giving rise to multinucleate protoplasts. A linear relationship was found between protoplast volume and number of nuclei per protoplast.

1. Introduction

2. Materials and Methods
Monokaryotic mycelium of Schizophyllum commune (strain 699) was grown in liquid minimal medium (Wessels 1965) for 2 days at 24 °C. Protoplasts were liberated by using a lytic enzyme system from Trichoderma viride in the presence of 0.5 M MgSO₄ and 0.05 M Na-
maleate (pH 5.8) according to the method of de Vries and Wessel (1972). The enzymatic
digestion took place in shallow layers to ensure aerobic conditions. After 11 hours of
digestion, the mixture was centrifuged (20 minutes, 1,400 g) in order to remove undigested
hypha and wall material. The top layer, mainly containing highly vacuolated floating
protoplasts, was collected and resuspended in a small volume of clear supernatant fluid.
The protoplast suspension was then incubated in a shallow layer at 24 °C and samples
were taken at intervals.
Samples were fixed in either a mixture of methanol and acetic acid (3 : 1, v/v) at 5 °C for
1-3 days or in 1% (w/v) mercuric chloride in methanol at —30 °C for 2-4 days. The
fixed protoplasts were concentrated by centrifugation and a few drops of a suspension
were brought on a slide. After evaporation of the fixative the protoplasts were stuck to
the slide by dipping it in a 30 times diluted 1:1 (v/v) mixture of fresh egg white and
glycerin and drying in air.
Nuclei were stained by the HCl-Giemsa procedure. Hydrolysis was done in 5 N HCl at
room temperature for 20-40 minutes and staining was carried out in a solution containing
1% (v/v) Giemsa stain (Merck) in 0.006 M phosphate buffer (pH 6.8) for about 4 hours
at room temperature.
The concentration of native protoplasts was determined by counting in a haemocytometer
using phase-contrast microscopy.
Diameters of nucleate protoplasts were determined on Giemsa stained preparations.

3. Results and Discussion

After 11 hours of digestion of the mycelium with lytic enzymes 20-30% of
the released protoplasts contained one or more nuclei. Evidently, one
hyphal compartment of the monokaryotic mycelium of S. commune may
release several protoplasts only one of which has the nucleus. Strunk (1970)
reported similar findings for protoplasts of the dikaryotic mycelium of
Polystictus versicolor, most of which had no nuclei.
During digestion of the mycelium with lytic enzymes a number of protoplasts
acquired large vacuoles and increased in size, particularly under highly
aerobic conditions. These protoplasts floated in the digestion mixture and
could be collected as a top layer after centrifugation (de Vries and Wessel
1972). Since 50-70% of the floating protoplasts was nucleated, this provided
an easy method for enrichment of nucleate protoplasts.
When the floating protoplasts were incubated in the presence of wall-lytic
enzymes, preventing wall regeneration, their concentration decreased steadily
(Fig. 1). Because it was established that the loss of protoplasts was minimal
during the staining procedure, the percentages of nucleate and anucleate
protoplasts, as determined on stained preparations, could be used to calculate
their concentration in the protoplast suspension. Fig. 1 shows that the
decrease of the protoplast concentration was initially due only to disintegration
of anucleate protoplasts. After 9 hours of incubation large nucleate
protoplasts were almost exclusively left. During further incubation, nucleate
protoplasts also disintegrated.
Fig. 2 shows that during incubation of floating protoplasts in the presence
of wall-lytic enzymes, the percentage of multinucleate protoplasts of the