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Protoplasts from Neurospora crassa.

Protoplasts from yeast and molds are usually prepared by incubation of cells or hyphae with commercially available snail gut enzyme. However, in the case of Neurospora crassa, protoplast formation proceeds slowly and is incomplete. De Vries and Wessels (1973 J. Gen. Microbiol. 73: 13) have shown that the cell walls of a number of molds contain constituents that cannot be hydrolyzed by the enzymes present in snail gut preparations. For complete digestion of cell walls there authors used exoenzymes produced by the mold Trichoderma viride when grown in a liquid medium with cell

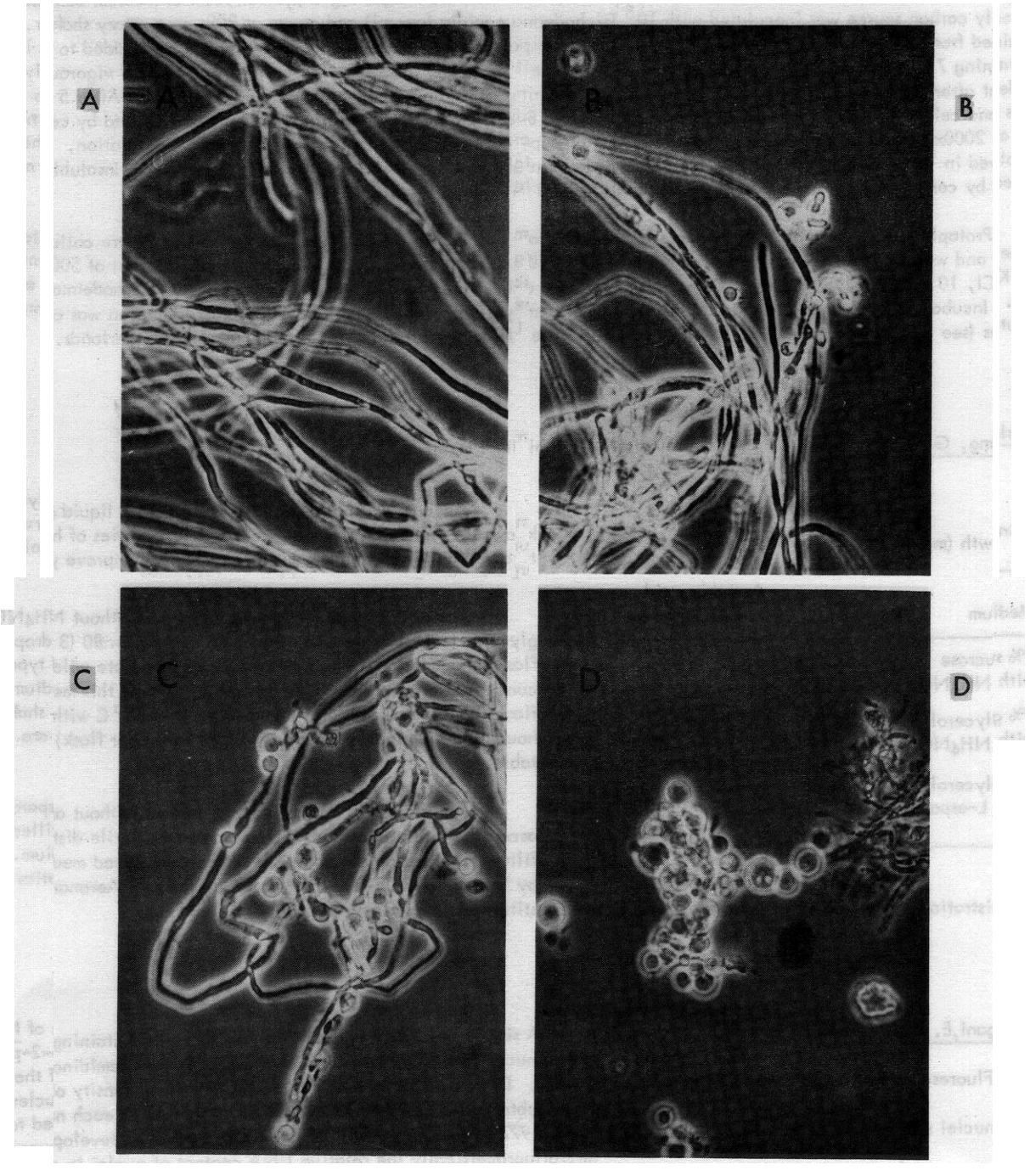

Figure 1. -- Phase contrast micrographs (magnification 500x) of Neurospora crassa hyphae and protoplasts before (A) and after incubation for 30 (B), 60 (C) and 90 (D) minutes with cell wall digesting enzymes from Trichoderma viride.

walls as a carbon source. We hove adapted this method for the isolation on a preparative scale of Trichodermo enzymes that can be used for the formation of protoplasts from hyphae of Neurospora crassa.

The growth medium for Trichodermo viride contained per liter: 2g KH_2PO_4, 1 g $(NH_4)SO_4$, 0.39 urea, 0.3g $MgSO_4 . 7H_2O$, 0.3g $CaCl_2$, 1 g bactopeptone and 1 ml of a trace element solution. The composition of the trace element solution was per 100ml: 50mg $FeSO_4$ $7H_2O$, 15.6mg $MnSO_4 . H_2O$, 16.7mg $ZnCl_2$, 20mg $CoCl_2$ and 0.1 ml 19% HCl. When glucose was used as the only carbon source, 5g per liter was added to the growth medium. With cell walls as carbon source, 50g of Neurospora cell walls (wet weight) plus 0.59 glucose were added per liter medium. The cell wall preparation was obtained as the 2000xg pellet of Neurospora hyphoe disrupted in a grindmill (H. Wiess et al. 1970 Eur. J. Biochem. 14: 75). This pellet was suspended in distilled water and rehomogenized in the grindmill. The washed cell walls were collected by centrifugotion. The washing procedure was repeated four times. For the production of cell wall digesting enzymes 100ml of medium containing glucose as the only carbon source was inoculated with 10^5 Trichodermo conidia (per ml) and grown at 30° on a rotary shaker. Conidia were obtained from solid grown cultures as described for Neurospora. After 24 hours the 100ml culture was added to a 10 liter bottle containing 7 liters of medium supplied with Neurospora cell walls and glucose. The culture was aerated vigorously and growthwas evident after two days as foam production (excessive foaming could be suppressed by adding antifoam). After 5 to 7 days of growth, cells and cell walls were removed by filtration through a Büchner funnel. The turbid filtrate was clarified by centrifugation for 10 min at 2000xg. The enzyme was precipitated from the supernatant with ammonium sulphate at 75% saturation. The precipitate was dissolved in 10 to 20ml distilled water and dialyzed overnight at 4° against 2 x 5 liter distilled water. Insoluble material was removed by centrifugotion and the enzyme preparation (100 to 300mg) was stored at -20° or lyophilized.

Protoplasts from Neurospora crassa were prepared from cultures in the early log phase. Hyphae were collected on a Büchner funnel and washed twice with ice cold distilled water. 10g hyphoe (wet weight) were suspended in 50ml of 500 mM sorbitol, 200 mM KCl, 10 mM $MgCl_2$, 0.1 mM EDTA, 50mM maleic acid, adjusted to pH 5.8 and 20mg of the Trichoderma enzyme preparation. Incubation was carried out at 30° in a 250ml erlenmeyer with gentle shaking. Protoplast formation was complete in 60-90 minutes (see Figure 1). - - - Lob. Physiol. Chem., State University Groningen, Groningen, The Netherlands.