

Preparations of Cell-Walls of Some Fungi

LITTLE is known of the qualitative and quantitative composition that may occur in the cell wall of fungi. Cochrane¹ has claimed that the most serious defect in our knowledge of cell-wall composition in the filamentous fungi is the fact that no 'pure' wall material is yet available. The major sources of error are the inclusion of extraneous substances in the preparation, and the loss of wall material during purification. Several workers have described results of the cell-wall composition based on X-ray diffraction patterns. Results from other methods (isolation of derivatives from a hydrolysate, microchemical tests, etc.) are usually in agreement with those of X-ray analysis, but are in most cases unsatisfactory.

This communication describes a method for the isolation of cell-walls of various moulds. Organisms were grown in Czapek medium shaking at 25° for 48 hr. avoiding sporulation. The resulting mycelium was then collected in a centrifuge and washed three times with water. After resuspension in water the mycelium was partially disintegrated by means of concentric ground-glass cones, washed five times and treated with ethyl ether for 1 hr. A serious difficulty occurred when attempting to disintegrate mycelium before treatment with ethyl ether. The resulting mycelium was washed with water and cell-walls were prepared by the method of Salton and Horne², by using the Mickle disintegrator. 10 ml. of the partially broken mycelium suspension were added to each cup of the machine and shaken with 10 ml. of grade 12 Ballotini glass beads for 4 hr. at room temperature. Breakage was followed by microscopic examination of lactophenol blue stained material.

After breakage, intact mycelium and debris were removed by centrifugation at 500g and the supernatant was transferred into a narrow glass tube (3 x 100 mm.) and the contents spun down at 1,000g. The sedimented material, containing unbroken cells, was discarded. The supernatant suspension was then centrifuged at 5,000g for 20 min.

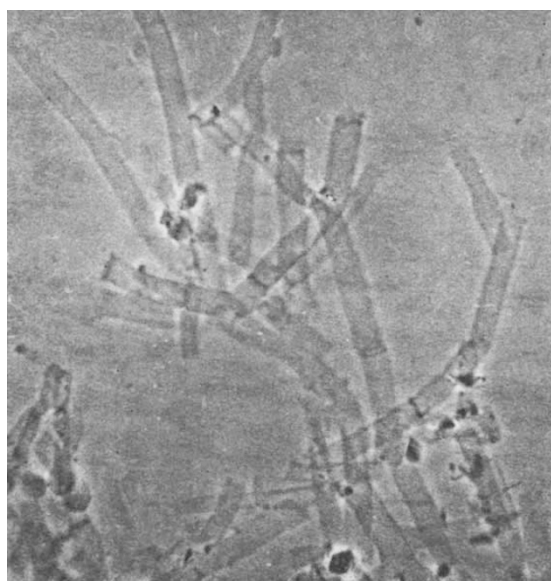


Fig. 1. Purified cell-walls of mycelium of *Aspergillus nidulans*

to sediment the cell-wall fraction. This material was digested first with crystalline trypsin and then with pepsin, as described by Villanueva³. The digest was centrifuged at 5,000g and washed once with 1 M sodium chloride solution, the precipitate being resuspended in water and thoroughly washed. The purity of the preparation was checked by light and electron microscopic examination.

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¹ Cochrane, V. W., *Physiology of Fungi*, 39 (John Wiley and Sons, Inc., New York, 1958).

² Salton, M. R. J., and Horne, R. W., *Biochim. Biophys. Acta*, 7, 177 (1951).

³ Villanueva, J. R., *Microbiol. Espanol.*, 13, 1 (1960).

Some Fossil Chelonian Fragments from Makapansgat

THE fossil chelonian material loaned to me by Prof. R. Dart, of the University of the Witwatersrand Medical School, for examination consists of 36 fragments from the early Middle Pleistocene grey breccia of Makapansgat Limeworks in the Transvaal. This fossil material was compared with complete and fragmented skeletal material of *Testudo pardalis babcocki*, *Kinyxs belliana belliana* and *Pelusios sinuatus*, all of which occur in the area to-day. The fossil fragments were all from large animals and none was referable to the genera *Chersina*, *Psammoplates*, *Homopus* or *Pelomedusa*, which were also available for comparison.

Twenty-four bone fragments were assigned to *Testudo pardalis*, representing a minimum of eight individuals, but only two fragments are definitely from the same specimen. Most of the material is derived from large animals (exceeding 12 in. in length and 20 lb. in weight) and the majority of the fragments are from the plastron, particularly the thickened gular area. The frequency of occurrence for the various bones is as follows :

Carapace	5th neural	1
	pleurals	4
	pygal	1
Plastron	epiplastra	9
	entropiastra	2
	hyoplastra	3
	hypoplastra	5
	xiphiplastra	3

One bone fragment consists of the gular section of the epiplastra of a moderate sized chelonian which may be *Kinyxs* sp. Unfortunately, there are no other fragments to establish the presence of this genus.

Three plastron fragments are assigned to *Pelusios* sp. They are from a large terrapin(s) comparable in size with *Pelusios sinuatus*.

Six small fragments could not be assigned to any form with confidence. No less than 22 of the 28 fragments identified were from the plastron.

The two terrestrial forms, *Testudo* and *Kinyxs*, are both widely used as food by man to-day, the former being prized because of its large size. The hinged terrapins (*Pelusios*) are aquatic animals, but are often caught on rod and line and are eaten by many Africans. There seems to be little doubt that the chelonian fragments dealt with here are from in-