à 250, 260 et 280 nm a permis l'estimation quantitative des composants en utilisant les coefficients d'extinction molaire donnés par MARKHAM[15].

L'établissement de courbes complètes d'absorption UV pour chaque composant isolé a complété son identification préalable sur la base du Rf et sa co-chromatographie avec des composés connus. En outre, deux analyses de contrôle de la technique avec du RNA de levure (Sigma) ont donné un rapport purines/pyrimidines moyen de 1,06 (1,00 selon ELSON et CHARGAFF[16]) et une somme G + C (voir plus bas) de 50,52.

Quatre séries successives de cultures d'*Allomyces* ont fait l'objet de 6 analyses de RNA pour les mâles et 5 analyses pour les femelles dont les valeurs moyennes sont présentées dans le Tableau II.

Aucune différence très significative n'est apparue entre la composition nucléotidique de la fraction saline du RNA de jeunes gamétanges mâles et celle des gamétanges femelles au stade de développement correspondant. Tout au plus pourrait-on remarquer la relative pauvreté de la femelle en acide adénylique et sa richesse en acide uridylique. Ses jeunes gamétanges contiennent d'ailleurs deux fois plus d'uracil(-uridine) libre que les organes mâles correspondants[4].

Tableau II. Composition nucléotidique[a] comparée du $RNA_{NaCl\ soluble}$ extrait des jeunes gamétanges mâles et femelles d'*Allomyces*

	Mâles	Femelles
Acide adénylique (adénine)	25,71 ± 1,05	24,15 ± 0,32
Acide guanylique (guanine)	30,17 ± 1,18	31,20 ± 1,47
Acide cytidylique	21,08 ± 1,54	20,25 ± 0,57
Acide uridylique	23,04 ± 1,16	24,40 ± 0,73
Acide guanylique + cytidylique	51,25	51,45
Purines/pyrimidines	1,27	1,24

[a] Moles/100 Moles de nucléotides.

Le rapport purines/pyrimidines s'est le plus souvent montré plus élevé dans les gamétanges mâles et, lors d'une analyse portant sur des gamétanges un peu plus mûrs (corps paranucléaires en organisation), nous avons obtenu une valeur de 1,21 pour le rapport mâle contre 1,13 seulement pour la femelle. A noter que ce dernier rapport est en bon accord avec celui trouvé dans des gamétanges femelles analysés par la méthode de séparation des nucléotides sur résine Dowex (1,15–1,16[4]).

Par contre, la somme acide guanylique + acide cytidylique (G + C) ne diffère pas entre mâle et femelle et, comme le RNA total de *Neurospora*[17] et de levure (voir ci-dessus), le RNA d'*Allomyces* se range dans la catégorie à GC faible[18].

Summary. Estimation of RNA and DNA by gamete in the unisexual strains of *Allomyces* showed the amount of RNA to be twice as much in the female as in the male. The amount of DNA was only slightly, though consistently, higher in the female. Comparison of NaCl-soluble RNA from young gametangia of the unisexual strains showed no appreciable difference in the nucleotide composition, although a tendency for a higher purine pyrimidine ratio was noticed in the males.

M. A. VISWANATHAN et G. TURIAN

Laboratoire de Microbiologie, Institut de Botanique générale, Université de Genève (Suisse), le 6 janvier 1966.

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Preparation of Spore Coats of *Fusarium culmorum*

Mould spores have been studied mainly from their physiological aspects and little has been done on the structure and composition of conidia. The main difficulty in this kind of study on the fungal cell wall results from the fact that no pure wall material is yet available. HORIKOSHI and ILDA[1] have recently studied the spore coats of unicellular conidia of *Aspergillus oryzae*, but reports on this subject are very rare. The method used to obtain isolated spore coats of *Fusarium culmorum* is described here. The work was undertaken in relation with investigations on germination and 'protoplast' formation from conidia of *F. culmorum*[2,3].

The culture of *F. culmorum* used in these experiments was obtained from the Colección Española de Cultivos Tipo and was maintained on potato dextrose agar by mass spore transfer. *F. culmorum* is characterized by the formation of extremely abundant macroconidia. Under proper conditions sporulation is so profuse that by suitable techniques masses of spores can be obtained substantially free from vegetative mycelium and other contaminants. Ungerminated spores have been considered as surrounded by an amorphous cell wall and a number of poorly defined structures in the cytoplasm. Multicellular macroconidia were harvested from 5-day-old cultures of the mould grown in Roux bottles on solid glucose-aspargine-yeast-extract medium[4] incubated at 26 °C. They were harvested by scraping with sterile glass beads after adding sterile distilled water.

In order to eliminate contamination from mycelium and from medium components, spore suspensions were

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passed through a coarse fritted-glass plaque 11C 1 Jena, without pressure. Spores were washed three times with water and appeared free from mycelium (Figure 1). After resuspension in water, the spores were treated in the Mickle disintegrator using glass beads (Ballotini No. 12), and samples were withdrawn at different times. After 12 h treatment, 98% of spores were broken. Several other methods for disrupting tissues have been used, but most of them have proved ineffective or they break the spore into too small pieces, as in the case of sonic vibration that makes difficult the selective recuperation of spore coats. Spore coats were collected by differential centrifugation by the following procedure: The contents of the tubes were allowed to stand until the glass beads settled out. The supernatant was decanted into a centrifuge tube and spun at 1000 *g* for 10 min. The sedimented coats were resuspended in distilled water and washed repeatedly by centrifugation until the supernatant became clear. The residue was the spore coat fraction and the purity of the preparation was at least 96–98% as determined by phase contrast microscopy. During the mechanical treatment spores were broken at both ends of the elongate cells, the contents going out through these holes. During this treatment the cross-walls of the spores were also disrupted, as can be observed in the electron micrographs (Figure 2) and the contents of the non-terminal cells liberated. The spore coats appeared to be free from cytoplasmic contents, as they were not stained with the ordinary cytoplasmic stains.

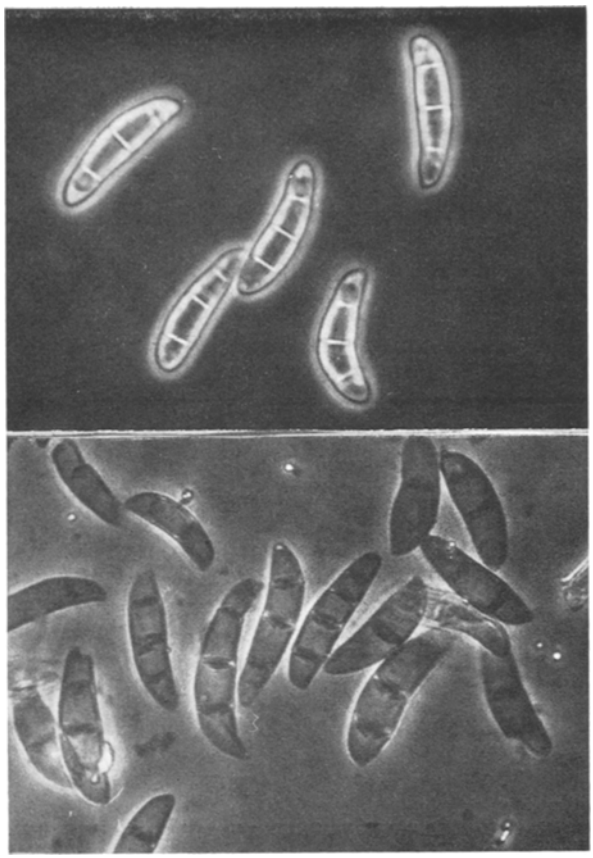

Fig. 1. Conidia of *Fusarium culmorum*: (top) before mechanical breakage; (bottom) after breakage showing partial damage of the empty spore coats.

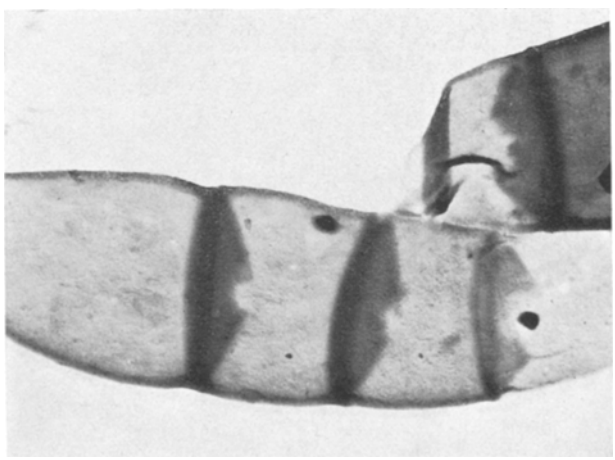

Fig. 2. Electron micrograph of the spore coat of *Fusarium culmorum*. The septa visible at the centre of the figure is believed to have been broken during the mechanical disintegration of the conidia.

The present study shows the spore septa as described by REICHLE and ALEXANDER[5]. They are composed of an external very hard and rigid frame, to which is attached a more or less rigid membrane holding a central pore, and that is disrupted during the treatment. Septa of the conidia seem to be an independent entity. We have found also that under certain conditions lytic enzymes[3] used in our laboratory to obtain mould protoplasts can digest most of the conidial cell wall of *F. culmorum*, leaving a residue of insoluble septa possessing a drum structure. Electron micrographs of isolated conidial septa did not show the presence of micropores described in the cross-walls of the mycelium of some fungi[6].

Spore coats observed under the phase contrast microscope show a crystalline aspect. Intact spores were refringent under these conditions, whereas spore-coat preparations were darker and transparent. The external surface of spore coats does not show any defined structure when observed in the electron microscope, although under certain conditions the presence of some fibrous structure on the external side of the walls was observed.

A comparison of the chemical composition of the spore walls of *F. culmorum* with those of normal mycelium of the same organism has recently been made[7,8].

Resumen. Se especifican las condiciones óptimas para la preparación de paredes celulares de macroconidios de *Fusarium culmorum*, utilizando el desintegrador de Mickle y se demuestra la pureza de las preparaciones mediante su examen al microscopio de fase y electrónico.

I. GARCÍA ACHA, J. P. G. BALLESTA,
M. J. R. AGUIRRE, and J. R. VILLANUEVA

Instituto de Biología Celular, C.S.I.C., Madrid-6 (Spain), December 27, 1965.

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