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COMMON CELLOPHANE WRAPPING AS SUPPORTING SUBSTRATE FOR THE CULTIVATION OF FUNGI AND OBTENTION OF PERMANENT MYCOLOGICAL PREPARATIONS

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SUMMARY

A standardization of the technique for the cultivation of both yeast and mycelial phases of fungi, on discs of plain cellophane wrapping, set on the surface of solid culture media, is proposed. The method affords the easy obtention of permanent mycological preparations.

INTRODUCTION

In medical as in general mycology, the species identification of any fungal agent is based primarily on micromorphological studies. The techniques currently employed for the preparation of slide or chamber cultures are very variable, as are their results, different investigators preconizing different procedures. In a general way, all of them are time-consuming and require great skill from the operator.

In a paper on methods for the study of moulds, FLEMING & SMITH [1 (*)], and again SMITH [7] in his book on industrial mycology, have included a technique for the cultivation of moulds on cellophane or other make of transparent viscose film, exclusion being made to cellulose acetate film. Surprisingly, widely adopted laboratory manuals such as the one issued by the U.S.D.H.E.W.[4] and REBELL & TAPLIN's [5], to name just two of them, never do quote this very practical technique, not to mention the countless investigations being published in the current scientific journals, which keep steadily to RIDDELL's [6] slide culture method.

In 1953, WINDLE TAYLOR, BURMAN & OLIVER [8] have described the use of a cellulose membrane-filter technique for the bacteriological examination of water. It was based on this particular investigation, and following the general line proposed by WINDLE TAYLOR et al.[8] for the isolation of pathogenic organisms from sewage effluents and polluted water by means of membrane-filters, that we have standardized a simple and very inexpensive procedure for the easy obtention of micro-colonies of fungal specimens, employing ordinary cellophane wrapping as supporting substrate.

The cellophane disc method may be summarized as follows:

MATERIAL AND METHODS

Sheets of ordinary cellophane wrapping are arranged in a pile with intercalated filter paper sheets, and 12 mm diameter discs are punched off by means of a punch chisel. The resulting stacks of cellophane/filter paper discs are packed into 13 x 115 glass tubes provided with a cotton plug, and autoclaved at 120°C for 15 to 20 minutes. The filter paper discs act merely as isolation pads to prevent adhesion between adjacent cellophane discs during sterilization.

The culture media employed in the present investigation were: Sabouraud's agar, potato-

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agar, and Fava Netto's. The cellophane discs are placed with sterile forceps on the surface of the chosen sterile culture medium, contained in 9 cm Petri dishes, under asseptic conditions. Each such culture unit may hold up to 20 discs. The cellophane sticks to the substrate readily. By means of a wire loop or a very fine glass pipette, a droplet of the inoculum consisting of spores or small bits of mycelia suspended either in sterile distilled water or 0.85% NaCl solution, is placed at the center of each cellophane disc. For didactic purposes, different known species may be seeded in the same culture unit. The lid of the Petri dish is fastened tightly with adhesive tape to prevent drying up. The specimens studied proceeded from the collection of the Department of Microbiology and Immunology of the Faculty of Bio-Medical Sciences of the University of São Paulo.

The development of the colonies is followed up by daily examination of the Petri dishes under a stereoscopic microscope. When the desired size and stage of development of the colony is attained, the growth may be interrupted. The material may be submitted to staining either directly or upon inactivation. Vapours from a formalin-soaked cotton placed laterally in the culture unit overnight will kill every culture.

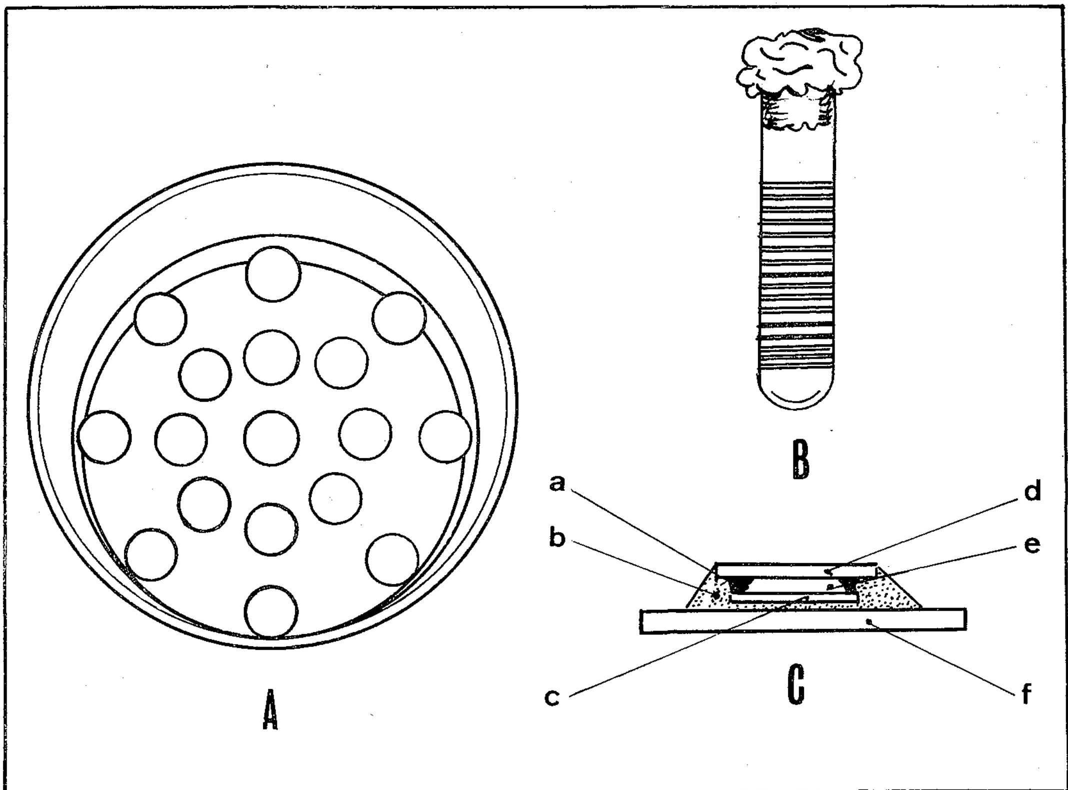

Fig. 1 — Diagrammatic representation of the main steps involved in the cellophane-disc technique for the cultivation of fungi and obtention of permanent mycological preparations.

A — Culture unit: Petri dish with sterilized cellophane discs overlaying the culture medium, ready for inoculation.

B — The 12 mm cellophane discs conditioned with intercalated filter paper pads in 13 x 115 glass tubes, ready for use.

C — Section through the final mounted slide obtained: a, dye; b, resin; c, cellophane disc; d, coverslip; e, fungal growth; f, microscopic slide.

One drop of the dye — AMMAN's lactophenol or its modified form, MÜKERJI's lactochloral (*) — is placed at the center of a coverslip; the cellophane disc supporting the fungal growth is dettached from the culture medium by means of a forceps, turned upside down and gently lowered upon the dye. Excess dye is absorbed by strips of blotting paper applied to the coverslip edges.

At the same time, one drop of synthetic resin (**) as recommended by KIRKPATRICK & LENDRUM [2, 3] for routine use in histology, is placed on a microscopic slide; the coverslip supporting the cellophane disc plus fungal growth is then transferred to the glass slide with a forceps, and set down right on top of the resin drop, the free surface of the cellophane disc touching the resin. A light pressure applied on the coverslip will eliminate most of the resin surplus, and the preparation is allowed to dry overnight. Any excess resin is then trimmed off with a razor blade. A diagrammatic representation of the main steps involved in the new technique, is shown in Fig. 1.

(*) In BRONTE GATENBY, J. & BEAMS, H. W. — The Microtomist's Vademecum. Seventeenth edition. London, J. & A. Churchill Ltd., 1950 (p. 209).

(**) HSR Microscopic Mounting Medium — HARTMAN-LEDDON, CO., Philadelphia, Pa., USA.

RESULTS AND COMMENTS

The method just described, provides a rapid routine diagnostic aid: in a matter of days, good preparations of the samples investigated can be obtained, with all the morphological characteristics of taxonomic importance well preserved. Figs. 2 through 5 show some of the fungi so far prepared by the cellophane disc technique in our laboratory: *Microsporum canis*, *Sporotrichum schenckii*, *Histoplasma capsulatum* and *Nigrospora sphaerica*. Furthermore, individual giant mould colonies may be raised in each 9 cm Petri dish and mounted between watchglasses, after fixation in formalin vapour, as permanent preparation for the study of colony morphology.

Fig. 2 — Four day culture of *Microsporum canis* (Sample 575, 8/1/1972), on a cellophane disc overlaying potato-agar. One week old preparation. Mükerji's lactochloral staining, 800 X.

Fig. 3 — Sixteen day culture of *Sporotrichum schenckii* (Sample 424, Patient MFG., 1972), on a cellophane disc overlaying Fava Netto's medium. Sixteen day old preparation. Amman's lactophenol staining, 400 X.

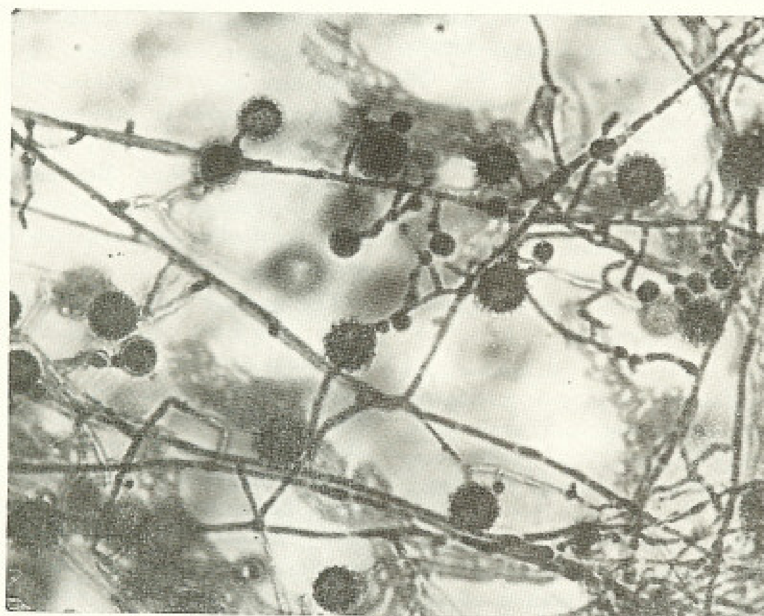

Fig. 4 — Fourteen day culture of *Histoplasma capsulatum* (Sample "Helio", patient HDS, 1972), on a cellophane disc overlaying Sabouraud's agar. Four month old preparation. Amman's lactophenol staining, 400 X.

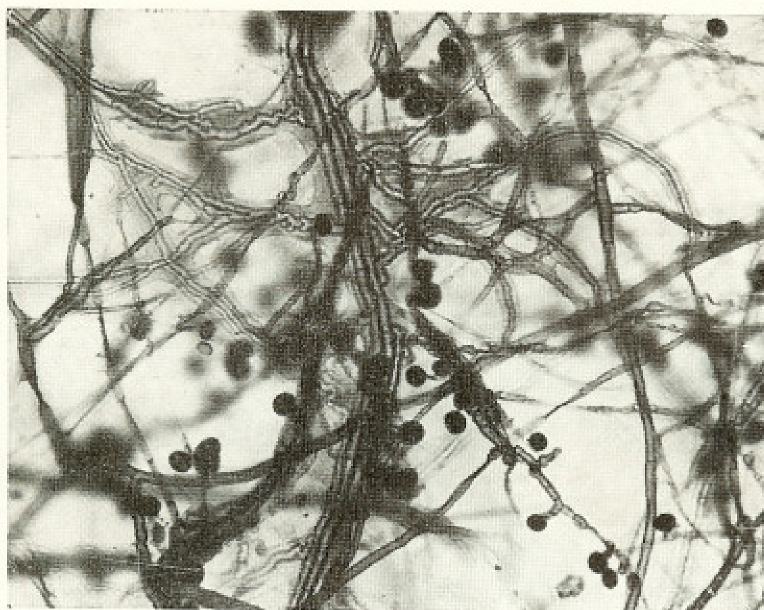

Fig. 5 — Fourteen day culture of *Nigrospora sphaerica* (Sample 278) on a cellophane disc overlaying Sabouraud's agar. Four month old preparation. No staining, 160 X.

For two decades now, RIDDELL[6] or SMITH[7] slide culture technique has been widely employed. Although providing a simple means for the observation of fungal growth and the developing structures *in situ*, as the cellophane disc technique does, it still requires a rather large manipulation of the material before getting to the final stained preparation, the adhering medium residues representing the main drawback of the method. In our technique, this last hindrance has been entirely overcome, as the cellophane peels off cleanly from the underlaying medium.

There is also a saving in glassware and in media preparation. In long-term observations, medium exhaustion and desiccation before completion of growth are hardly foreseen in our conditions, whereas they may come about in slide or chamber cultures. Aeration conditions are obviously adequate. As for the discs proper, we have not yet carried out any comparative studies regarding different qualities of cellophane wrappings; we should recommend, though, that it be thin and flexible. The preparation and conditioning of the discs in the way described have proved extremelly practical, the risks of contamination being minimal.

A further improvement in the staining procedure may be obtained by the use of supra-vital dyes. In preliminary experiments, we have attained fairly good preparations simply by allowing the culture-supporting discs to float over a supra-vital dye solution. This particular step requires further standardization.

The method may have different potentialities: for instance, based on the fact that the fungi growing on cellophane discs at the experimental conditions described, do get their nourishment from the underlaying medium by capillarity through the cellophane pores, it might be expected that, on the other hand, horizontal spreading of mutually inhibitory metabolites or invasive growth from contaminant organisms, may not occur or may be very restricted. Thus, the cellophane disc method may prove adequate for the isolation of pathogenic fungi directly from the specimen collected from the patient, which shall be next tried in our laboratory.

VOLCÁN G., G. S. — Common cellophane wrapping as supporting substrate for the cultivation of fungi and obtention of permanent mycological preparations. *Rev. Inst. Med. trop. São Paulo* **15**: 310-315, 1973.

RESUMEN

Cultivo de hongos sobre discos de papel celofan y obtención de preparaciones mycológicas permanentes

Se describe un método para cultivar hongos tanto en su forma miceliana como levaduriforme, sobre discos de papel celofan colocados em cajas de Petri que contienen medios de cultivo sólidos y húmedos. La técnica posibilita la obtención de láminas con colonias que pueden ser coloreadas y montadas con resinas sintéticas, lo que permite conservarlas permanentemente.

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