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The « Water Cultivation » of Pathogenic Fungi

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Over twenty years ago I gave an account in the Journal of Tropical Medicine and Hygiene (42, 255) 1939, of an experiment carried out in the Mycological Laboratories of the London School of Tropical Medicine.

Original experiment. On July 5th 1938, twelve tubes of sterile distilled water were inoculated with the following fungi : *Candida krusei* Cast., *C. albicans* Robins var. *pinoyi* Cast., *C. tropicalis* Cast., *C. pseudotropicalis* Cast., *C. macedoniensis* Cast., *Geotrichum rotundatum* Cast., *G. matalense* Cast., *G. asteroides* Cast., *G. rugosum* Cast., *Epidermophyton floccosum* Hartz, *Cladosporium mansoni* Cast., *Aleurisma (Acladium) castellanii* Pinoy.

The distilled water tubes were inoculated from glucose agar cultures, care being taken that particles of the glucose agar were not transferred to the liquid. The tubes were sealed at the flame and kept at room temperature until July 10th, 1939, a period of one year and five days. The tubes were opened and after shaking, inoculations were made from each tube into glucose agar. Growth developed in all the glucose agar tubes within the normal time and the macroscopic appearance of the cultures was normal.

The Candidae were passed through the series of carbohydrates I used at the time, namely, glucose, levulose, mannose, maltose, galactose, saccharose, lactose and inulin. The fermentation characters had not undergone any change.

The strain of *E. floccosum* inoculated into distilled water was an old laboratory strain which had become partially pleomorphic several years previously, being fluffy but still showing a certain amount of characteristic canary-yellow colour. The cultures were made on glucose agar after twelve months, maintenance in distilled water, showing the same partial pleomorphism with some characteristic yellow colour present.

From the amount of sediment in the inoculated tubes the impression was obtained that several of the fungi must have grown slightly. This was certainly the case with *Cladosporium mansoni* and some species of *Candida*.

Since then the experiment has been repeated more than once using the fungi mentioned above, and in addition the following : *Sporotrichum anglicum* Cast., *Trichophyton rubrum* Cast. and a number of other species of *Trichophyton* and *Microsporon*, including *Tr. concentricum* Blanchard var. *tropicalis* Cast. and var. *indicum* Cast.; also *Coccidioides immitis* Rixford and Gilchrist, strain *me-*

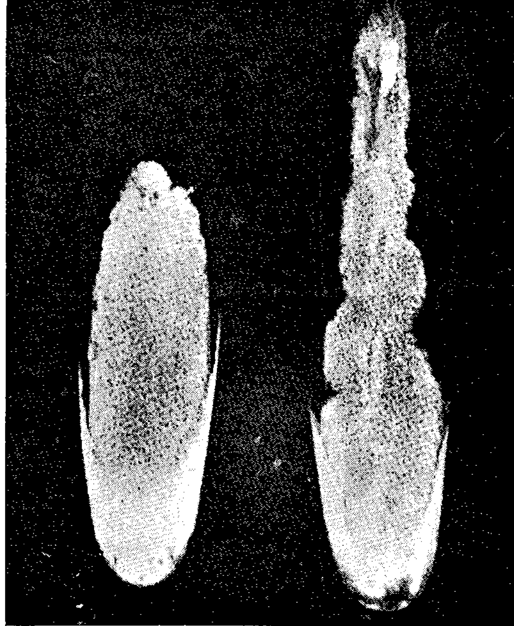
taeuropeus Cast., *Blastomyces dermatitides* Gilchrist and Stokes, strain *tulanensis* Cast., *Cryptococcus neoformans* Sanfelice, *C. neoformans* strain *hondurianus* Cast., *C. ater* Cast., *C. genitalis* Cast. The results have been constantly the same. After twelve months all the fungi were alive and grew quite well in glucose agar, producing colonies exactly like the original ones, and still has the same morphological and biochemical characters, moreover it was found that they remained viable far longer than a year.

The very simple original technique has been rendered even simpler in recent years by discarding the sealing of the inoculated tubes at the flame. Ordinary tubes containing 6.0, 8.0, or 10 ml of sterile distilled water, plugged with cotton wool like ordinary tubes of broth and other media are used. They are inoculated with a large inoculum and kept in the laboratory at room temperature (in hot countries it is advisable to use rubber caps to prevent evaporation of liquid). When using a large inoculum, the transferance of a little of the glucose to the tube of distilled water is practically unavoidable, but the amount of glucose so added is so minute that it is not likely to influence sensibly the growth of the fungus or facilitate the development of pleomorphism.

The above experiments have led me to devise a very simple procedure for maintaining pathogenic fungi, especially yeasts and dermatophytes, in mycological collections, by cultivation in sterile distilled water. Tubes of sterile distilled water are inoculated and left at room temperature for twelve months. They are plugged with cotton wool or sealed at the flame.

After one year subcultures are made from them on to glucose agar to see whether the fungi are alive and have maintained their original characters. From these glucose agar cultures a new series of distilled water cultures are made, and a year later the process is repeated. This method dispenses with the necessity of frequent subculturing and makes unnecessary the use of lysolitic procedures which are much less successful with mycetes than with bacteria, some mycetes, e.g. *Cladosporium (Aurobasidium) mansoni* frequently dying out in the process. Another advantage of the method is that it seems largely to prevent pleomorphism, although of course it does not cure it once it has developed: a pleomorphic strain inoculated into sterile distilled water remains pleomorphic.

APPLICATION TO SOME BACTERIA. As stated in a lecture to the New York Academy of Sciences (Castellani, 1962) not only very numerous mycetes are viable and capable of growth in sterile distilled water for over a year, but a certain number of bacteria, especially of the Enterobacteriaceae, are capable of doing so. Among



(I) (II)

(I) Four-days-old glucose-agar culture made from water culture of *Candida krusei* 12 months old. (II) Four-days-old glucose-agar culture made from stock glucose-agar culture of *Candida krusei* (same strain).

them are *Salmonella typhosa* and *paratyphosa*, *S. schotmuelleri*, *S. asiatica*, *Morganella columbensis*, *Proteus morgani* and *Cloaca cloacae*.

Conclusion.

My researches appear to have demonstrated that practically all the pathogenic fungi of man excluding the Actinomycetes (they are no longer considered fungi) remain viable and capable of growing in sterile distilled water, for a least a year, and possibly, in fact, almost indefinitely if the evaporated water is replaced annually.

Based on these results a simple method has been devised for maintenance pathogenic fungi in mycological collections, the reliability of which has been recently confirmed by Mungelluzzi and Castagnetta (1962) and its simplicity and usefulness have been emphasised by Benedek (1962), who in his paper on the subject in *Mycopathologia et Mycologia Applicata*, has written « Castellani's « Water culture » method for microscopic fungi was reexamined and confirmed in its every detail. It is an ideal method for, at least, the smaller culture collection, in order to avoid continuous, short-term subculturing. »

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