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EX AEDIBVS ACADEMICIS IN CIVITATE VATICANA

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THE CULTIVATION OF PATHOGENIC FUNGI IN STERILE DISTILLED WATER

ALDO CASTELLANI
Pontifical Academician

SVMMARIVM — Ex Auctoris indagationibus omnes fungi, qui homini sint patogeni — exceptis actinomycetis, quae iam nemo fungis adnumerat — posse vivere et crescere videntur, saltem per unum annum, in aqua distillata sterili; quodsi aqua semel in anno subrogetur, posse sine ullo fere temporis limite vivere.

Ex hac observatione nova eaque facilis ratio inventa est fungorum patogenorum conservandorum in mycologicis collectionibus.

Over twenty years ago I gave an account in the *Journal of Tropical Medicine and Hygiene* (August 1st, 1939) of an experiment carried out in the Mycological Laboratories of the London School of Hygiene and 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* Robin var. *pinoyi* Cast., *C. tropicalis* Cast., *C. pseudotropicalis* Cast., *C. macedoniensis* Cast., *Geotrichum rotundatum* Cast., *G. matalense* Cast., *G. asteroides* Cast.,

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G. rugosum Cast., *Epidermophyton floccosum* Harz, *Cladosporium mansoni* Cast., *Aleurismo* (*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 appearances of the cultures were normal.

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

The strain *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*.

Further researches

Since then the experiment has been repeated more than once, using the fungi mentioned above, and in addition the following: *Sporotrichum anglicum* Cast., *Glenospora lanuginosa* Cast., *Trichophyton rubrum* Cast., and many other spe-

cies of *Trichophyton* and *Microsporon*, including *Tr. concentricum* Blanchard var. *tropicale* Cast., and var. *indicum* Cast.; also *Coccidioides immitis* Rixford and Gilchrist, strain *metaeuropeus* Cast., *Blastomyces dermatitidus* 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 had the same morphological and biochemical characters. Moreover, it was found that they remained viable far longer than a year in fact, apparently indefinitely, if the evaporated water was replaced.

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 distiled 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 it is practically unavoidable to transfer a little of the glucose agar to the tube of distilled water, but the amount of glucose so added is so minute that it is not likely to influence the growth of the fungus sensibly 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 maintain-

ed their original characters. From these glucose agar cultures a new series of sterile 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 bacteria

As stated in a lecture to the New York Academy of Sciences (Castellani, 1962) not only very numerous mycetes remain viable and capable of growth in sterile distilled water for over a year, but a certain number of bacteria, especially of the family *Enterobacteriaceae,* are capable of doing so. Among 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 which are no longer considered fungi, remain viable and capable of growing in sterile distilled water for at least a year, and apparently almost indefinitely if the evaporated water is replaced annually.

Based on these results a simple method has been devised for maintaining 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 the *Mycopathologia et Mycologia Applicata* has written: « Castellani's 'Water Culture' method for microscopic fungi was re-examined 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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