A PHOTOGRAPHIC METHOD FOR DETERMINING APICAL GROWTH IN FUNGAL HYPHÆ

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It is well recognized that growth in fungal hyphæ is only apical and considerable evidence to this effect has been adduced by Smith (1923) in the case of a variety of fungal forms. The technique developed by him of sowing fungal spores on a cover slip coated with agar medium and inverting the latter on a van Tieghem cell, growth measurements being made at intervals of time, has obvious shortcomings in its applicability to measure growth rate of rapidly growing fungi; the fastest growth obtained by Smith (1923) in the case of *Rhizopus nigricans* was less than 100 microns per hour which is too low a figure for a rapidly growing fungus like *R. nigricans*; it is not clear whether this was due to the low nutrient content of the medium, or the influences of the van Tieghem cell, or due to the intrinsic nature of that particular isolate itself. Nonetheless the limitations of van Tieghem cell and its tendencies to exaggerate anaerobic influences on fungal growth are evident. The present technique was developed to measure growth rate in vitro aiming chiefly at providing tangible evidence of apical growth in fungal hyphæ for Laboratory demonstration purposes, on somewhat similar pattern to the photographic method described by Vyvyan (1924) for measuring growth rates of leaves. As far as the author is aware no photographic evidence is available of the microscopic growth of fungal hyphæ and its complementary changes during growth (branching, etc.).

*Rhizopus nodosus*, a very fast growing strain isolated from cotton seed by the author (Venkatram, 1950), was employed in this investigation. The fungus was inoculated in the centre of sterile agar plates of standard potato dextrose medium; 10 cm. ‘K’ Petri dishes were selected for this purpose since they were convenient to handle, comparatively shorter and of good transparency to facilitate direct microscopic observation; the agar medium was filtered thrice to obtain good clarity. Growth measurements were made six hours after inoculation using Zeiss × 10 objective and screw type adjustable eyepiece micrometer. One single hypha was brought into the
Figs. A–E show growing hyphae of *Rhizopus nodosus* (×60). The time interval between exposures *seriatim* from A to B to C to D to E was $2\frac{1}{2}$, $1\frac{1}{2}$, $3\frac{1}{2}$ and 4 minutes respectively.
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microscopic field so that the hyphal tip came flush with the 0 scale of the micrometer; thereafter the number of divisions traversed after suitable intervals of time were noted or vice versa the time taken to cover the distance of 5 micrometer divisions was determined by a stop-clock arrangement. Due to the very rapid growth of the fungus (in this case being nearly 60 microns per minute), accurate camera lucida drawings of the branching habits, etc., were not possible, consequently photomicrographs of the growth of the fungal hyphae were taken at suitable intervals of time using a Zeiss Miflex camera attachment. This arrangement enabled correct focussing of the fungal hyphae at different stages of growth, to be achieved, just prior to the photographing. Illumination of the microscopic field was so manipulated as to give a good picture with exposure of one second on Ilford H.P. 3 Plates. Very satisfactory results were obtained by this method as is seen from Plate XVI.

It was observed that although branching occurred freely, the position of these branches on the micrometer scale never changed (Plate XVI), thus proving that hyphal growth was entirely apical. Since the conditions provided herein were comparable to those for determining the radial growth of fungi in any desired medium, rate of growth measured by this technique would serve as a suitable index to compare growth rates between fungal forms in a definite medium or inversely of one form in different media, at a particular time. In actual practice it was found that figures obtained in microns of the growing hyphal tip converted into mm. then multiplied by 2 (factor 2 gives diametrical spread in two directions being a colony) and calculated against time approximately gives colony diameter in mm. which is the conventional unit of growth measurement of growing fungi in culture media.

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References

