place only in MS+IAA medium. Shootlets continued to grow after rooting, 4–6 leaves appeared shortly.

Plantlet regenerating potential of hypocotyl and cotyledon explants of several *B. oleracea* varieties have earlier been reported[1], but these explants from *B. oleracea* var. *caulorapa* did not respond to the media tested. Chakraborty and Roy[2] reported commercial production of cabbage plantlets from auxillary buds. Our results suggest that large scale *in vitro* plantlet production of this variety is also possible and can be favourably exploited for genetic engineering and clonal propagation.

7 May 1988

1. Zee, S. Y. and Johnson, B. B., In: *handbook of plant cell culture*, Vol. 3, (eds) P. V. Ammirato, D. A. Evans, W. R. Sharp and Y. Yamada, Macmillan, New York, Collier, London, 1984 p. 227.
2. Chakraborty, S. and Roy, S. C., *Indian J. Exp. Biol.*, 1987, **25**, 61.
3. Mascarenhas, A. F., Handre, R. R., Nadgir, A. L., Durga Durga, Barve, H. and Jagannathan, V., *Indian J. Exp. Biol.*, 1978, **16**, 122.
4. Bajaj, Y. P. S. and Mahapatra, D., *Indian J. Exp. Biol.*, 1987, **25**, 161.
5. Murashige, T. and Skoog, F., *Physiol. Plant.*, 1962, **15**, 473.

IMMOBILIZED *SPOROTRICHUM THERMOPHILE* PRODUCES CELLULASE

ALKA SINGH, REETA GOEL and B. N. JOHRI
Department of Microbiology, G. B. Pant University of Agriculture and Technology, Pantnagar 263 145, India.

THE concept of immobilized cell-dependent process is relatively old[1,2]. The wide applicability of this concept to modern bioreactors is, however, a more recent innovation and to date has been used in large scale manufacturing for only single-step reactions[3]. In the present study, spores of *S. thermophile* (active producer of cellulase[4,5]) have been immobilized to produce extracellular components of cellulase. The *in situ* growth was examined by light as well as electron microscopy.

A culture of *S. thermophile* was obtained from the culture collection of the department and maintained in YpSs agar. It was cultivated using the procedures and media described by Canevascini[5].

Three different matrices, viz. agar, polyacrylamide and alginate were used to entrap the spores of *S. thermophile* by established techniques[6–8]. Agar beads were prepared using 10^{-6} spores ml^{-1} in 2.5% agar powder. For *in situ* growth beads were incubated in 100 ml medium[5] at 45°C for 120 h. Since agar beads showed higher cellulase activity and better mycelial growth, they were used for further experimentation. Cellulase estimation was done according to IUPAC method[9].

For light microscopy hand-cut sections of the beads were mounted in glycerine and examined

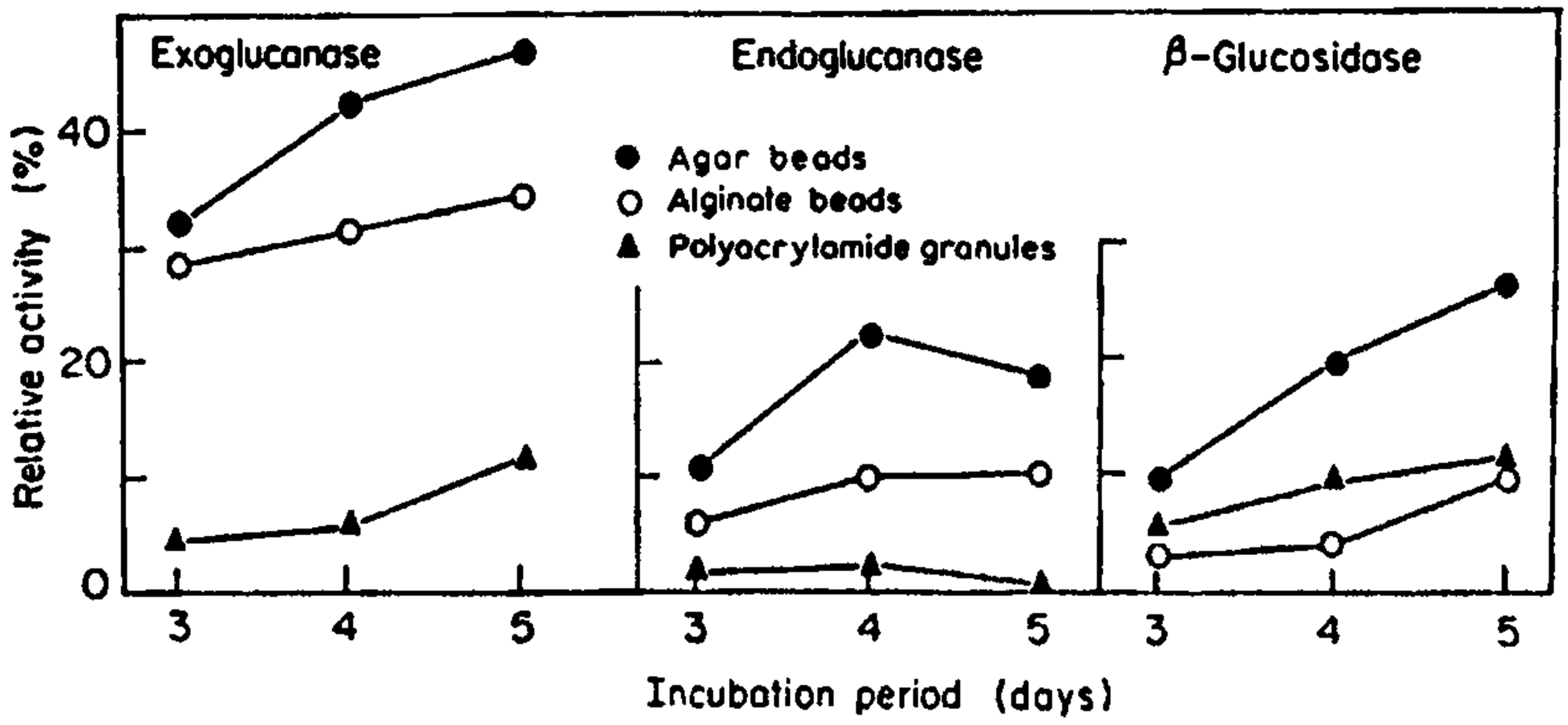


Figure 1. Screening of various supports for immobilization of *Sporotrichum thermophile* and cellulase production.

under bright field and or phase contrast using an Olympus microscope.

For electron microscopy, beads were prefixed in 3% glutaraldehyde with 0.2 M phosphate buffer, post fixed with 2% OsO_4 for 2 h and dehydrated by gradial acetone. For infiltration and embedding, araldite was used. Sections were examined at 12,000 ×.

The physical stability of the beads as also the growth and cellulase productivity of *S. thermophile* were affected by the type and concentration of the support used. 2.5% agar beads yielded the best results (figure 1). Figure 2 shows the light micrographs of beads taken after 0, 24 and 120 h of *in situ* growth. After 24 h incubation the conidiospores germinated well in the beads (figure 2a, b). Gradually it formed a mycelial mass around the periphery and throughout the porous beads (figure 2c, d). Proliferation of extensive hyphae was visible in the electron micrographs which also exhibited that the mycelia were normal (figure 3a, b).

The mycelial filaments extended throughout the solid matrix. This indicated that the turgor pressure of the growing mycelial tip was sufficient to allow penetration of the solid agar matrix.

A few reports concerning the growth of bacterial and yeast cells in immobilized matrices are available[10]. The filamentous fungi grow by linear elongation of mycelial filaments. Deo *et al*[11] described *in situ* germination of conidia of *Penicillium urticae* immobilized in K-carrageenan beads, using scanning electron microscopy. These growing

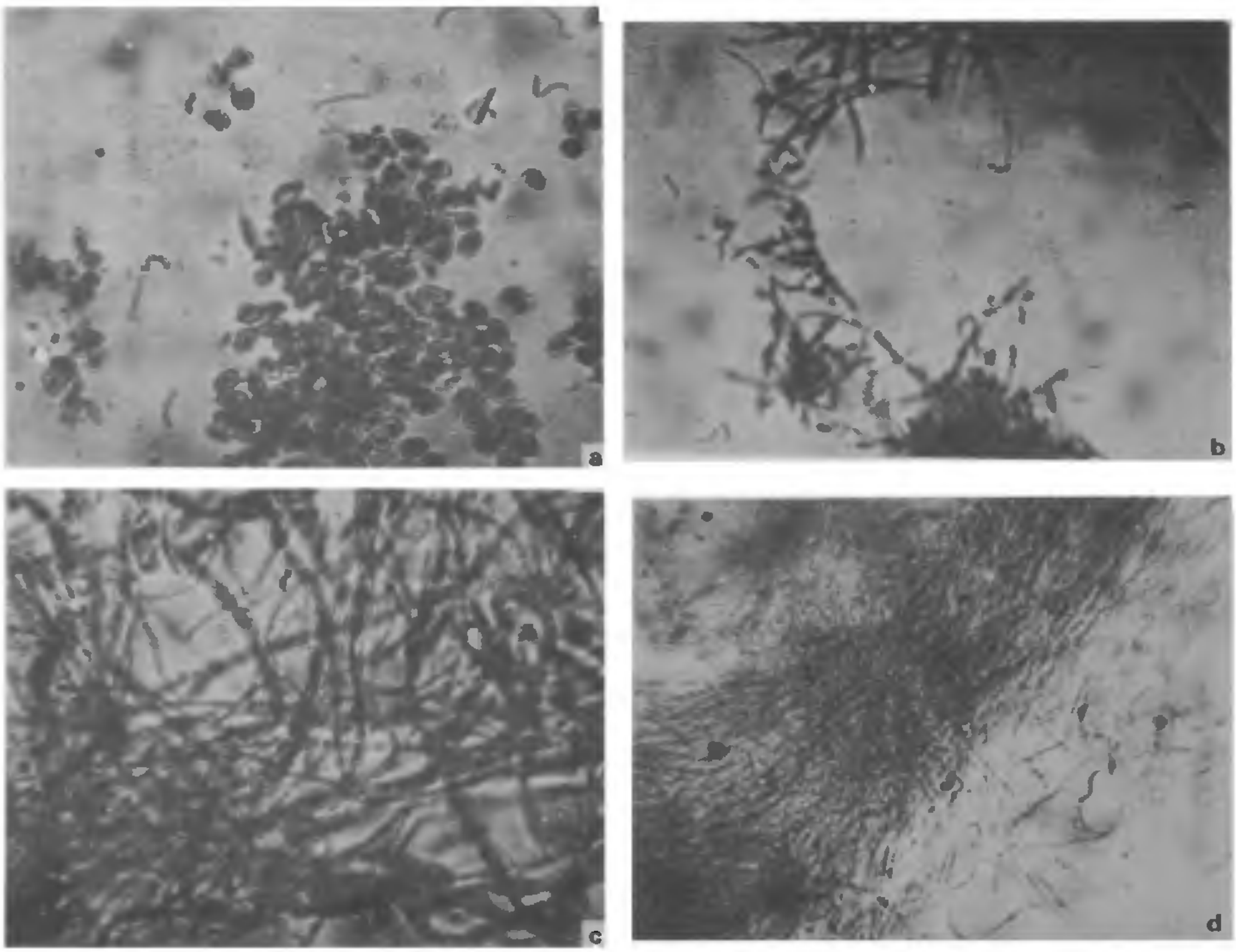
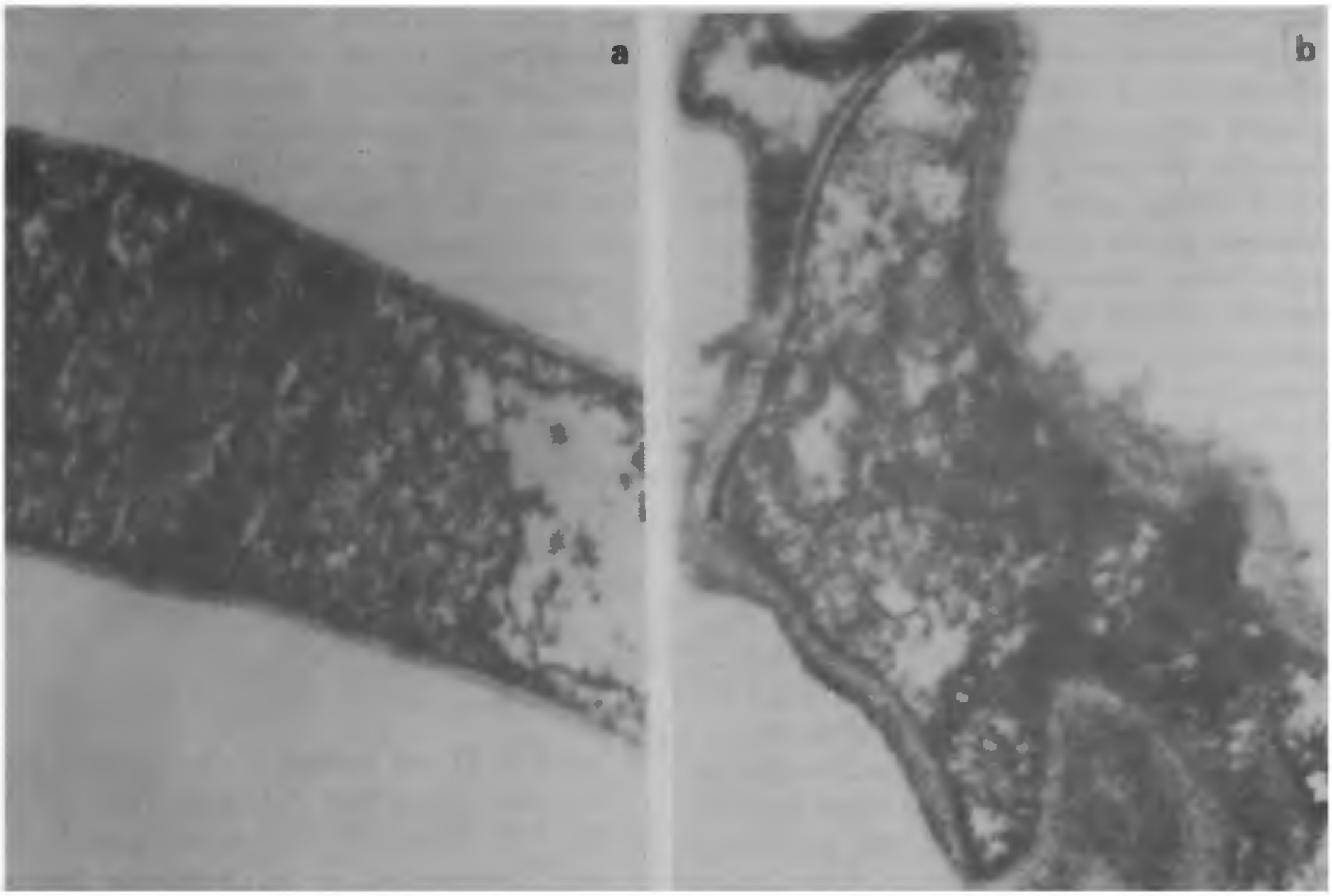


Figure 2a–d. Light micrographs showing the *in situ* germination of *Sporotrichum thermophile*. **a.** Agar entrapped spores; **b.** Germinated spores after 24 h of incubation; **c.** Mycelia after 72 h of incubation, and **d.** After 120 h of incubation.



filaments, as shown in this study, have the potential to invade the entire matrix, with the extent of biomass formed being dependent upon diffusibility of the nutrients from the bulk medium into the matrix, the space available for mycelial growth within the matrix and the ability of a growing mycelial tip to penetrate it.

One of the authors (AS) acknowledges assistantship awarded by the university.

4 June 1988; Revised 9 August 1988

1. Venkatsubramanian, K. and Veith, W. R., *Progr. Ind. Microbiol.*, 1979, **15**, 61.
2. Cooney, C. L., *Science*, 1983, **219**, 728.
3. Chibata, I., Tosa, T. and Takata, I., *Trends Biotechnol.*, 1983, **1**, 9.
4. Coutts, A. D. and Smith, R. E., *Appl. Environ. Microbiol.*, 1976, **31**, 819.
5. Canevascini, G., Coudray, M. R., Rey, J. P., Southgate, R. J. G. and Meier, H., *J. Gen. Microbiol.*, 1979, **110**, 291.
6. Kuo, W. Y. and Polack, J. A., *Biotechnol. Bioeng.*, 1983, **25**, 1995.
7. Mosbach, K. and Larsson, P. O., *Biotechnol. Bioeng.*, 1970, **12**, 19.
8. Kierstan, M. and Bucke, C., *Biotechnol. Bioeng.*, 1977, **19**, 387.
9. *Measurement of cellulase assay*, Commission on Biotechnology, 1985, IUPAC.
10. Wada, M., Kato, J. and Chibata, I., *Eur. J. Appl. Microbiol.*, 1980, **10**, 275.
11. Deo, Y. M. and Gaucher, G. M., *Biotechnol. Lett.*, 1983, **5**, 125.

CHROMOSOMAL VARIABILITY IN *CHARA CORALLINA* WILDENOW UNDER CULTURE CONDITIONS

B. R. CHAUDHARY and ANJALI DAS
Centre of Advanced Study in Botany, Banaras Hindu University, Varanasi 221 005, India.

THE alga was collected from Sarnath, Varanasi during November 1987. Individual plants, with intact root systems, were planted into the soil in