13 August 1982

1. Bansal, H. C., Santha, I. M. and Bhaskaran, S., *Barley Genet. Newslett.*, 1979, **9**, 13.
2. Johnson, R. R., Willmer, C. M. and Moss, D. N., *Crop. Sci.*, 1975, **15**, 217.
3. Shannon, J. G., and Reid, D. A., *Crop. Sci.*, 1976, **16**, 347.

BASIDIOCARP PRODUCTION BY *TERMITOMYCES MICROCARPUS* (BERK. AND BR.) HEIM IN CULTURE

A. B. De
Department of Botany, Burdwan Raj College, Burdwan, 713 104, India

Termitomyces microcarpus (Berk. & Br.) Heim is an edible mushroom of India[1]. In the present paper the formation of typical basidiocarp of *T. microcarpus* in culture is described.

The sporophores of *T. microcarpus* were found to grow on soil in Government Reserved Forest, Burdwan, West Bengal, India. Spore deposit was taken from one of these sporophores at the central position on a sterile agar plate. A colony of dikaryotic mycelia was developed from this spore deposit. Within 7 days the colony became 5 cm in diameter. Inocula of uniform size (5 mm in diameter) were obtained by punching out agar discs from advancing zone of this colony. One inoculum was lifted aseptically in each slant containing 10 ml of 2.5% malt agar medium. These slants were exposed to 0, 6, 12, 18 and 24 hr of light (intensity 1,000 lux) every day and then incubated in complete darkness. Incubation temperature was 28 ± 2° C.

Complete darkness inhibited stipe formation. Similar observation was made by some previous workers[2-5]. The slants exposed to 6, 12 and 18 hr of light showed initiation of primordia of basidiocarp within 35–40 days of inoculation but continuous light inhibited basidiocarp initiation. Alternating light and dark periods were required for basidiocarp initiation[3,4]. Further development of the primordia was stopped in the slants exposed to 6 and 12 hr of light but stipe elongation and pileus formation was noticed in slants exposed to 18 hr of light. Manachere[6] also observed that fruit-body development in *Coprinus congregatus* Bull. ex Fr. was only possible if cultures were subjected to suitable light and dark periods.

Initially the stipes were at right angle to the wall of the culture tubes which soon turned towards the mouth of the tubes and grew vertically. The stipes elongated rapidly and reached a length of 2–3 cm within 24–30 hr when their further growth was stopped and their apical ends became knob-like. Within the next 48–72 hr each knob-like apex transformed into a fully-developed pileus (figure 1) producing gills underside. The pileus produced in artificial medium measured 1–1.5 cm in diameter, stipe 2–3 cm long and 0.2–0.5 cm in diameter. Cultural sporophore had shorter stipe and slightly smaller pileus than the corresponding structures of natural sporophores but all the anatomical structures including hyphal elements, basidia, basidiospores and cystidioles produced in natural sporophores were also produced in cultural sporophore.

The present investigation shows that unlike some previous reports[4,7,8] CO_2 accumulated within the closed culture tubes due to respiration of *T. microcarpus*, did not prevent stipe elongation and pileus formation. Instead, light was a limiting factor

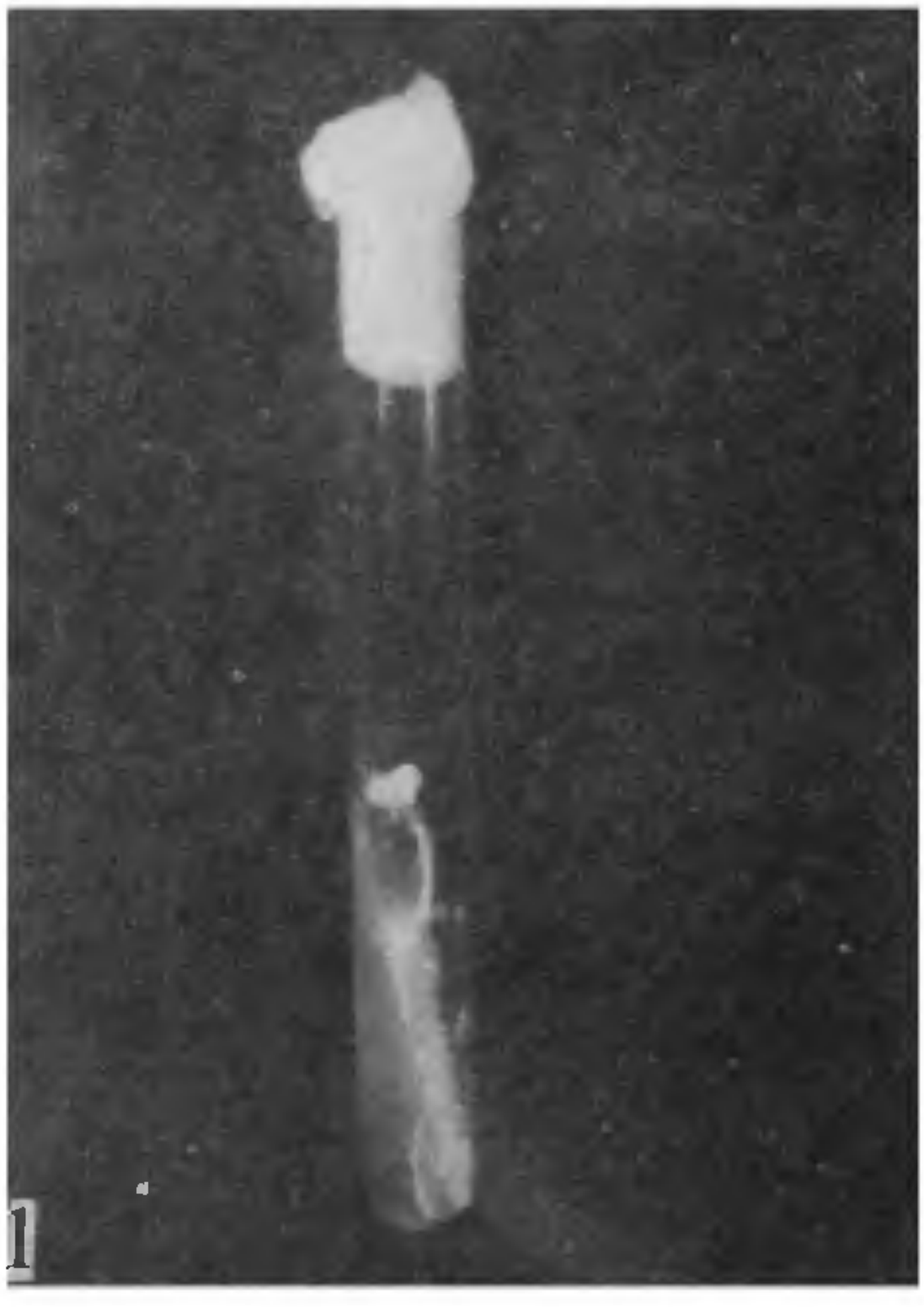

Figure 1. Typical basidiocarp of *Termitomyces microcarpus* (Berk and Br.) Heim. formed in culture tube (× ½).

because sporophore was produced only when a period of 6 hr of darkness was preceded by 18 hr of light. In the case of *Coprinus congregatus* Robert[9] reported that light period following the inductive dark-period could be concerned with the translocation of carbohydrates and proteins from stipe to the cap which facilitated cap maturation. But in the present study the nature of substances supplied by the mycelium during the elongation phase or maturation phase is not known.

The requirement of suitable light and dark period for pileus formation may also be connected with the fact that substances necessary for maturation of cap are synthesized in two steps—the first step requires light and the second step is inhibited by light or *vice versa*. Further investigation is needed to identify the translocated compounds and to indicate their steps of synthesis.

The author is greatly indebted to Dr Anjali Roy of Visva-Bharati University, Shantiniketan for her constant encouragement and to the Council of Scientific and Industrial Research, New Delhi, for a fellowship.

19 August 1982; Revised 2 December 1982

1. Bose, S. R. and Bose, A. B., *Sci. Cult.*, 1940, **6**, 141.
2. Bigelow, H. E., *Mycologia*, 1970, **62**, 203.
3. De, A. B., *Curr. Sci.*, 1978, **47**, 641.
4. De, A. B. and Roy, A., *Curr. Sci.*, 1978, **47**, 472.
5. Robert, J. C., *Trans. Br. Mycol. Soc.*, 1977, **68**, 389.
6. Manachere, G., *Annales des Sciences Naturelles, Botanique et Biologie Vegetale*, Paris, Ser. 12, **11**, 1.
7. McLaughlin, D. J., *Mycologia*, 1970, **62**, 307.
8. Banerjee, S. N. and Bakshi, B. K., *Curr. Sci.*, 1944, **13**, 102.
9. Robert, J. C., *Trans. Br. Mycol. Soc.*, 1977, **68**, 379.

EXTRACELLULAR PRODUCTS OF THREE SPECIES OF *ALTERNARIA*

P. BHARATHI, M. SUNITA, B. R. RENUKA AND M. VINAYA KUMAR
Department of Botany, Osmania University, Hyderabad 500 007, India.

THE taxonomy, physiology and distribution of *Alternaria* species are well-known but meagre information is available on the chemical constituents of the culture filtrates[1,2]. Based on the amino acid composition, it has been suggested[2] that *A. solani* is not closely related to either *A. triticina* or *A. alternata*. Few qualitative differences have also been observed[2] between the amino acids, organic acids and sugars in the mycelium of *A. triticina* and *A. alternata*, providing biochemical support to the suggestion that *A. triticina* may be an ecotype of *A. alternata*. In algae biochemical studies are known to help in recognising species of certain genera[3-5].

Three species of *Alternaria*, *viz.*, *A. brassicola* (Schw.) Wilt, a pathogenic form, *A. alternata* (Fries.) Kressler, a mild pathogen (isolated from *Bassica oleraceae* L. leaves) and *A. humicola* Oudemans, isolated from Hyderabad soil (pH = 8) were selected and the culture filtrates analysed for soluble proteins, total phenols, lactic acid and IAA.

The test fungi were maintained on potato sucrose agar medium, and grown on Czapek's Dox liquid medium (with 3% sucrose) and incubated for 15 days at 27° ± 2° C. Three replicates were maintained for each species and at the end of the incubation period the mycelium was separated by filtering through Whatman No. 1 filter paper. The culture filtrate was centrifuged at 3000 rpm for 5 min. This filtrate (25 ml) was refluxed with 80% ethanol for 10 min and the refluxed material was used to estimate total phenols[6]. Seventy five ml of the filtrate was used to estimate soluble proteins[7], lactic acid[8] and IAA[9].

Species of *Alternaria* differed significantly in the extracellular products, produced in culture filtrates (table 1). All the three species failed to produce IAA in culture. With a few exceptions, all fungi concerned with lactic acid production belong to the family *Mucoraceae* and chiefly to the genus *Rhizopus*[10]. Emerson and Cantino[11] have shown the production of lactic acid by *Blastocladia pringsheimii*. The ability to form lactic acid from hexose sugars is a common property of all biological systems, but it is surprising that it is a rarity in fungi[10].

TABLE 1

Some chemical constituents in the culture filtrates of three species of Alternaira (μ/ml)

Constituents	*A. alternata*	*A. brassicola*	*A. humicola*
Total phenols	13.6	45.6	61
Protein	96	14	10
Lactic acid	2.8	4.1	21
IAA	nil	nil	nil