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Bacterial Metabolites trigger Sporophore Formation in *Agaricus bisporus*

THE beneficial effect of one fungus on another was first reported by Heald and Pool[1] and has since been noted frequently. It was soon discovered that the contaminating organism supplied growth factors which the other organism failed to synthesize. *Agaricus bisporus* produces abundant sporophores in natural soil but not in aseptic environments. This suggests that certain microorganisms in soil trigger the formation of sporophores of *A. bisporus*. Fungi, bacteria, actinomycetes and yeasts were therefore isolated from the test soil and the role of these in the formation of sporophores was studied. It was interesting to find that only the presence of certain bacteria stimulated the formation of sporophores of *A. bisporus*. Using these bacteria or their metabolite(s), the production of mature sporophores of *A. bisporus* was achieved for the first time in aseptic environments.

The formation of sporophores of *A. bisporus* was studied in autoclaved soil (15 pounds/inch^2 for 48 h), gamma sterilized soil ($2{\cdot}5 \times 10^6$ rads of gamma ray for 48 h), and in soil extract medium[2]. The sterility of both the soils was confirmed on potato dextrose agar. Soil (10 g) or soil extract agar medium (20 ml.) was transferred to a 9 cm Petri dish. The soil moisture was adjusted to 40 per cent water holding capacity. Before inoculation the bacterial cells, growing on soil extract agar medium, were suspended in sterile water, and the suspension was centrifuged at 1,000*g* for 5 min to sediment the spores and to remove any water soluble metabolites. The spores were then suspended in 20 ml. of 1 mM potassium phosphate buffer at *p*H 6·5 or double distilled water. Each medium was inoculated with 2 ml. of bacterial suspension. The Petri dishes were incubated in the dark at 21° C. Primordia of sporophores appeared on the surface of medium as pin heads in 6 to 10 days and after 15 days produced mature fruiting bodies. Table 1 clearly shows that the presence of bacteria influenced the formation of sporophores, which was also dependent on the kind of medium; autoclaved soil and soil extract medium were superior to gamma sterilized soil. In general, *Arthrobacter terregens*, *Bacillus megaterium* and *Rhizobium meliloti* stimulated abundant production of mature sporophores of *A. bisporus*.

We have examined further the question of whether the formation of sporophores of *A. bisporus* was dependent on the presence of bacterium on or in its metabolite because Zentmyer[3] reported that the association of *Chromobacterium violaceum* was indispensable for the production of sporangia of *Phytophthora cinnamomi*. Bacterial colonies were suspended in sterile water, shaken vigorously for 5 min, passed through a 'Millipore' filter (0·45 micron pore size), and 2 ml. of filtrate was added to each medium. The control plates received 2 ml. of sterile water. The results showed that the initiation of sporophores was actually dependent on bacterial metabolite(s), for no sporophores were produced in the control. The present study concludes that soil bacteria (Table 1) produce some metabolite(s) that trigger the formation of sporophores of *A. bisporus*.

Table 1. PRESENCE OF DIFFERENT BACTERIA ON SPOROPHORE FORMATION OF *A. bisporus*

Bacteria	Autoclaved soil	Gamma sterilized soil	Soil extract
Bacillus megaterium	+ + +*	−	+ + +
B. cereus	+ +	+	+
Arthrobacter terregens	+ + + +	+ +	+ + + +
Rhizobium meliloti	+ + + +	−	+ + + +
R. leguminosarum	+ +	−	+ +
Azotobacter vinelandii	+ +	−	+
Control	−	−	−

* Index of sporophore formation: + (1–2); + + (3–5); + + + (6–10); + + + + (11–15).

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Prevalence of *Leptotrombidium deliense*, the Scrub Typhus Vector, in the Eastern Himalayas

MALARIA apart, scrub typhus is the most important hazard to the health of troops operating in many parts of South-East Asia. This is well documented in the medical history of the Second World War[1,2]. As a result of the Chinese aggression in 1962 the eastern Himalayas has assumed great military importance to India. Scrub typhus is known to be endemic in the entire range of the Himalayas, but systematic surveys of the various regions of the Himalayas where troops are likely to operate have not so far been made. I report here a survey of scrub typhus vectors in the eastern Himalayas.

During July 1966–May 1967 I surveyed in the State of Sikkim and Darjeeling and Jalpaiguri Districts of West Bengal up to altitudes of 3,840 m for *Leptotrombidium deliense* (*Trombicula deliensis*), the well known vector of scrub typhus, and for other trombiculids and ectoparasites. In all, 573 wild rodent and insectivore hosts of thirteen species were trapped and examined. Of the 283 rodents, 62 per cent were *Rattus rattus*, 13 per cent *Bandicota indica* and 6 per cent *B. bengalensis*; and all but one (*Tupia glis*) of the 290 insectivores were *Suncus murinus*: 357 (62 per cent) of the host specimens were infested with trombiculid larvae. Forty-nine were used as a source of seeding material for breeding an *L. deliense* colony at the base laboratory at the Armed Forces Medical College, Poona. The other 308 specimens yielded over 24,000 trombiculid larvae; 9,000 of these were examined and classified. *L. deliense* formed 22 per cent, *Leptotrombidium* spp. 13 per cent, *Gahrliepia* spp. 64 per cent, and *Schongastia* spp. 1 per cent. *L. akamushi*, a close relative of *L. deliense* but very uncommon in the trombiculid fauna of India[3], was found in the survey.

L. deliense larvae were recovered from all the areas surveyed including the high altitude areas: Kalimpong