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Ethylene Production by Axenic Fruiting Cultures of *Agaricus bisporus*

D. A. WOOD AND J. B. W. HAMMOND*

Glasshouse Crops Research Institute, Littlehampton, Sussex, BN16 3PU, United Kingdom

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Axenic cultures of *Agaricus bisporus* were used to show that the rise in ethylene production during fruiting derives from its own metabolism.

Recent work has shown a correlation between fruiting and ethylene production during the life cycle of the cultivated mushroom *Agaricus bisporus* (Lange) Sing. (4). However, the cultures were nonaxenic. The ethylene formed at fruiting could, therefore, have originated from the mycelium of *A. bisporus*, microorganisms in the culture medium or in the layer of peat and chalk ("casing") used to induce fruiting, or from the composted wheat straw (3).

We used cultures with a sterile, activated charcoal "casing" over axenically grown mycelium in compost (2) to determine whether the peak of ethylene production during fruiting derives from the metabolism of *A. bisporus* or from other microorganisms.

Cultures of *A. bisporus* strain D621 (Darlingtons Ltd., Angmering, Sussex) were grown and fruited axenically. Two experiments were carried out as follows. Glass jars of 1-liter capacity containing 100 g (fresh weight) of compost were sterilized by autoclaving at 120°C and 1.1 kg/cm^2 for 1 h. The compost was then inoculated with mycelium. After 2 to 3 weeks at 25°C, the colonized compost was covered ("cased") with a 1-cm layer of sterile, activated charcoal granules (British Drug Houses, Ltd.). The charcoal had been previously sterilized by dry heat at 160°C for 3 h and was subsequently moistened with sterile distilled water. The jars were each aerated with humidified, sterile, filtered air at a flow rate of 250 ml/min and kept at 20°C. Three types of controls were used and aerated in the manner described: (i) empty jars; (ii) jars with uninoculated, autoclaved compost; and (iii) jars containing uncased, autoclaved compost inoculated with *A. bisporus* mycelium. There were four replicates of each treatment. A Subaseal closure was inserted into a tube passing through the sealing bung of each of the jars to allow sampling of the gas atmosphere. Hyphal aggregates of about 2-mm diameter were normally found in the casing layer 5 to 10 days after it had been applied. Mature fruiting bodies developed from these aggregates 7 to 15 days after appearance of the aggregates.

For ethylene measurements by gas chromatography, the airflow was stopped and each jar was sealed by clamping the inlet and outlet tubes. Ethylene was allowed to accumulate for 5 h, and then a 1-ml sample was taken through the Subaseal closure with a hypodermic syringe. The sample was injected into a stainless-steel column (720 by 5 mm) containing Porapak S molecular sieve (Waters Associates [Instruments] Ltd., Stockport) and run at room temperature. The carrier gas was hydrogen/nitrogen (1:1) flowing at 2 liters h^{-1}. A flame ionization detector was used, and ethylene was identified by co-chromatography. For quantitative analysis, the chart peak height was compared with that obtained from a standard ethylene-air mixture.

After the cultures had fruited, samples (ca. 5.0 g) of the compost and activated-charcoal layers were removed with a sterile spatula. These were mixed with 10 ml of sterile saline (0.9%, wt/vol), and serial 10-fold dilutions were prepared. Samples (0.2 ml) of the original suspension and the dilution series were plated onto malt agar, nutrient agar, and PPLO broth agar. The plates were incubated for 5 days at 25°C and examined for fungal and bacterial colonies.

The production of ethylene from the flasks was monitored at approximately 6-day intervals during the vegetative stages, but fruiting cultures were sampled daily. Ethylene was detected in both the control and experimental jars, but an increase in its production was only found in cultures in which fruiting bodies developed (Table 1). The low level of ethylene detected in the control jars throughout the experiment (Table 1) may have been due to contamination of the air from rubber tubing and bungs in the apparatus, and, in the uncased control, which contained compost and mycelium, to a low level of ethylene production during mycelial colonization of the medium. In

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TABLE 1. *Ethylene production (nanoliters per hour) in control and experiment jars during axenic growth and fruiting of Agaricus bisporus*[a]

Prepn	Time after inoculation (days)		
	20	41	45
Empty jars	0.52	ND[b]	ND[b]
Uninoculated, autoclaved compost	0.065	0.195	0.195
Inoculated, uncased colonized compost	5.20	2.08	1.30
Cased, inoculated compost jar no.			
1	3.90	40.04	2.60
2	3.90	2.21	33.80
3[c]	2.60	1.30	2.60
4[c]	1.60	1.43	1.30

[a] Values are the mean of four replicates for the control treatments and individual values for the jars containing sporophores.
[b] ND, Not detectable.
[c] No sporophores formed.

none of the controls did the ethylene rise above the background levels throughout the experiment.

Fruiting did not occur synchronously between replicate "cased" cultures but, nevertheless, a rise in ethylene production always coincided with the development of one or more fruiting bodies in a particular jar (Fig. 1). Our results agree with previous work (4) in that maximum ethylene production occurred at stages 4 to 5 of fruiting (1) as the gill color was changing from pink to brown. Ethylene production then declined as the fruiting bodies sporulated and senesced. This pattern of ethylene production was found in both the experiments carried out. Plating of compost and casing samples from cultures bearing fruiting bodies showed that no other organisms capable of growth on the three recovery media were present. It has been shown (4) that fruiting bodies are not responsible for the ethylene peak, and our results indicate that it is produced by *A. bisporus* rather than by other members of the compost microflora. Therefore, the evidence from our experiments, together with the data of others (4), suggests that the mycelium of *A. bisporus* is the source of the increase in ethylene production during fruiting.

It is of some interest that a peak of ethylene

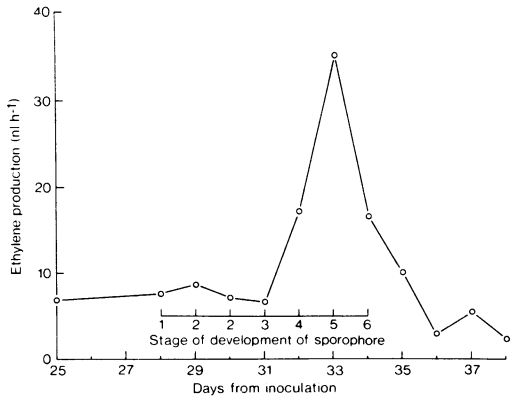

FIG. 1. *Production of ethylene in an axenic fruiting culture of Agaricus bisporus. Stage of development is expressed on a scale of 1 to 7 (1). Cultures were cased with sterile charcoal 16 days after inoculation.*

production is found to be restricted to a particular stage of fruiting development, and that this ethylene derives from the supporting mycelium. Previous work (4) showed that removal of a fruiting body just before its full expansion did not change the profile of ethylene production. These and earlier results (4) show that the burst in ethylene production is restricted to fruiting cultures (Table 1), indicating that fruiting bodies directly influence the metabolism of their connecting mycelia. Further work will be necessary before the route of ethylene synthesis and its significance in the life cycle can be determined.

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