Carbon dioxide and Ethylene Levels during Incubation and Fruiting Stages on Sawdust-Based Culture of Lentinula edodes

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Original Article

Carbon dioxide and Ethylene Levels during Incubation and Fruiting Stages on Sawdust-Based Culture of *Lentinula edodes*

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Abstract

The production of volatiles from the sawdust-based culture of *Lentinula edodes* was monitored during mycelial growth and fruit body first flush for 100 days. Carbon dioxide and ethylene production was recognized in relation to mycelial growth and fruit body development. These two volatiles were released with similar pattern. It was seen that high rates of both volatiles production occurred on days 20 to 30 of fully colonized stage, and day 60 of fruit body veil break stage.

Key words : shiitake; *Lentinula edodes*; carbon dioxide; ethylene, sawdust-based culture.

1. Introduction

Lentinula edodes (Berk.) Pegler is the most abundant cultivated mushroom in Japan, and it occupied the second most edible fungi in the world. Recently, the cultivation method on a sawdust-based substrate has made rapid increase instead of traditional log wood cultivation. Two phases are observed on the sawdust-based culture method: a vegetative mycelial growing phase, followed by a reproductive, fruit body forming phase. Various volatile compounds were produced in both of these two phases, and influenced mycelial growth and fruit body development.

The production of carbon dioxide and ethylene are commonly measured to observe the physiological activities of harvested cultural products. Long and Jacobs (1974) considered the involvement of volatile substances in the control of *Agaricus bisporus* development, and importance of carbon dioxide has been clearly demonstrated: low concentrations enhance and high concentrations suppress initiation, and carbon dioxide also enhances the subsequent elongation of the stipe. The ethylene and a number of volatile substances including acetaldehyde, acetone, ethanol and ethyl acetate have been identified as products of *A. bisporus* during both the vegetative phase and the reproductive phase (Lockard and Kneebone, 1962; Ward *et al.*, 1978).

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Production of ethylene on sawdust-based cultures of *Pleurotus ostreatus* (Yamanaka, 1982) and *Lentinula edodes* (Nakasawa *et al.*, 1978) was reported, respectively. Ethylene is produced by living, higher plant cells as part of normal metabolic activity (Ilag and Curtis, 1968). In higher plants, ethylene is a naturally produced growth regulator controlling many aspects of development. A number of other unsaturated hydrocarbons such as acethylene and propylene will also modify plant growth in a similar way, but at much higher concentration.

With the view that similar controls may exist in *L. edodes*, I have examined the production of gases such as carbon dioxide and ethylene during life cycle of *L. edodes* on sawdust-based culture growing in controlled conditions of laboratory equipment.

2. Materials and Methods

2.1. Microorganism and culture conditions

Pure culture of *Lentinula edodes* (Berk.) Pegler IFO 7123 was obtained from the Institute for Fermentation (Osaka, Japan). This strain has been grown in a potato dextrose agar medium (PDA, Difco Laboratories, Detroit, MI, U.S.A.).

The composition of the Henneberg medium was as follows: glucose, 50 g; KNO_3, 2 g; $NH_4H_2PO_4$, 2 g; KH_2PO_4, 1 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; $CaCl_2$, 0.1 g in 1L of distilled water. All chemicals used were analytical grade or biological grade, and purchased from Wako Pure Chemical Co. (Osaka, Japan).

Cultures were grown on a sawdust-based substrate, consisting of *Quercus mongolica* sawdust and various ingredients shown in Table 1 with water added to give a final moisture content of 63 %. Polypropylene bags were filled with the substrate (1 kg wet wt., ϕ 20×15 cm) then were autoclaved at 120℃ for 1 hr, cooled and through spawned with 10 g sawdust spawn. The bags were placed in a controlled environment for incubation. The bags were incubated at 20℃ for vegetative growth for 60 days. At the end of the incubation period on day 60, the plastic bags were removed and the colonized substrates were transferred to a production room, in which temperature was maintained at 17℃ and relative humidity was kept at 90 % throughout the experiment.

2.2. Analytical methods

For measurements of volatiles, each culture was set in a test bottle (ϕ 25×28 cm), and closed for up to 6 hours with a silicon stopper (Fig. 1). The accumulated volatiles were sampled for gas chromatographic analysis (Turner, 1975). One milliliter samples of the gas phase were withdrawn with a gas tight micro syringe (Gas Tight Syringe MS-GAN 250, Itoh, Tokyo, Japan) from the head space of the sealed test bottle containing cultures.

The gas sample was analyzed by a gas chromatograph system (Hitachi 163) fitted with an 1 m × 3 mm glass column containing 80 to 100 mesh silicon dioxide (Unibeads

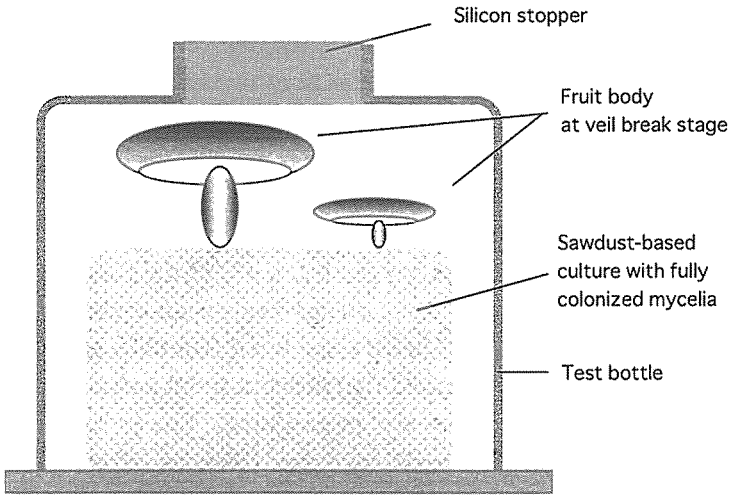

Fig. 1. Diagrammatic representation measuring carbon dioxide and ethylene of the sawdust-based culture of *L. edodes*. This is the fruit body development veil break stage on 70 days after inoculation. Test bottle size is ϕ 25 × 28 cm.

1S, Gaskuro Kogyo Co, Tokyo, Japan) at a temperature program of 40℃, 10℃/min to final temperature of 160℃. A hydrogen flame ionization detector was used. Helium was used as a carrier gas with a flow rate of about 30 ml/min. Chromatographic peak area measurements were made with a Chromatopac C-R1B (Shimadzu Industry Co., Japan). The gases were identified with authentic standard gases (Gaskuro Kogyo), and this certified gas standard was used for proportional calibration. Values for ethylene are presented as ng/g dry weight/hr. The carbon dioxide was analyzed with the same GC using a thermal conductivity detector. Values for carbon dioxide are presented as mg /g dry weight/hr.

Samples were tested in duplicate, and each experiment was done at least three times with similar results.

3. Results and Discussion

3.1. Mycelial growth and fruit body development

The sawdust-based culture was characterized as follows: The mycelia grew as a white vegetative colony for 10 days. The colony was sufficiently well-established to cover the surface of culture on day 20, then promoted the formation of the white, dense mycelial coat with white-dots on day 40. The mycelial coats took on a brownish pigmentation, and the bumps formed continuously on day 65. Until the 70th day the primordia barely increased in size, then they began to grow rapidly. During the 70th to 80th days the fruit bodies were growing and maturing.

3.2. Carbon dioxide evolution

A rapid increase was observed in carbon dioxide concentration in the mycelial fully colonized stage on day 20 (Fig. 2). After 20 to 40 days, carbon dioxide concentration decreased and began to a steadily increase. Then carbon dioxide concentration increased rapidly after fruiting treatment on day 60. The peak on the day 75 corresponds to the fruit body veil break stage.

Heartrot fungi can grow in a nearly anaerobic atmosphere containing high levels of carbon dioxide. Concentrations of oxygen below 1% of the volume of gases in tree trunks are common (Jensen, 1969), and carbon dioxide concentrations in intact wood range from 2% to 6%, and up to 15% in decaying wood (Hintikka and Korhonen, 1970). Hintikka and Korhonen (1970) reported that heartrot fungi are more tolerant of high carbon dioxide concentration than are saprot fungi. Lower carbon dioxide is a prerequisite for fructification in basidiomycetes (Sietsma *et al.*, 1977). It is well established that a surplus of carbon dioxide leads to repression of differentiation in fungi such as *Schizophyllum, Penicillum* and *Agaricus* (Sietsma *et al.*, 1977; Graafmans, 1973; Long and Jacobs, 1974). Inadequate gas exchange, resulting in depletion of oxygen and increase in carbon dioxide concentrations, probably was the primary cause of the inhibition observed in saturated and very wet culture. Compatible mating of *S. commune* carried out in sealed chambers showed good vegetative growth and clamp-connection formation but fruiting was markedly inhibited. Gas mixtures of

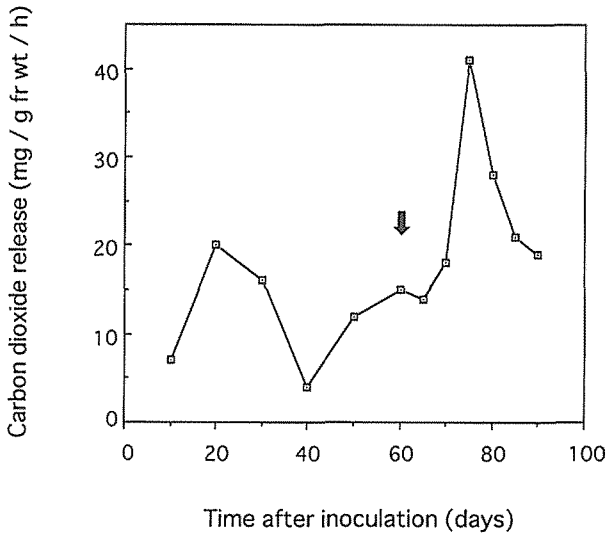

Fig. 2. Production of carbon dioxide in sawdust-based culture during mycelial growth and fruit body development. Mycelial growth phase is from day 0 to day 60, and fruit body development phase is day 60 to day 90. The arrow indicates fruiting treatment on day 60; (the fully colonized mature cultures were transferred from incubation room (20℃) to production room (17℃, 90% RH).

air-carbon dioxide (95:5) severely restricted the fruiting process when applied during mating or before the formation of fruit body primordia. It is proposed that respiratory carbon dioxide plays an important role in the regulation of form of *S. commune* (Niederpruem, 1963).

Under aerobic conditions, decomposition of wood by micro-organisms releases carbon dioxide and metabolic liquid. Water vapor, like carbon dioxide, is a product of fungal respiration. Both oxygen consumption and carbon dioxide evolution have been measured to determine decomposition rates of many types of plant litter in the laboratory (Howard and Howard, 1974; Bunnell, 1977). The carbon dioxide plays important roles in commercial production of mushrooms, such as *A. bisporus* and *L. edodes*. Many wood decay fungi grow more rapidly in elevated carbon dioxide concentrations, up to 10% to 15%. In contrast, *A. bisporus* is inhibited by carbon dioxide concentrations above 2% (Jensen, 1969). Other metabolic functions are variously affected by carbon dioxide concentrations, including enzyme activity, reproductive functions, and pigmentation.

3.3. Ethylene evolution

Ethylene was evolved from sawdust-based culture during mycelial growth and fruit body development. High rates of ethylene formation was observed in cultures containing various ingredients such as rice bran, wheat bran, and corn powder with the rapid growth rate of colonies (Table 1). The pattern and timing of peak of ethylene concentration was similar to the results of carbon dioxide concentration (Fig. 3). Ethylene production increased during colonization of the culture then declined from day 30 to day 60. A peak of ethylene release was observed on day 70 of the fruit body veil break stage. Although ethylene production rose during the early expansion of the fruit body, it declined to a low level on the senescent stage.

Table 1. Ethylene production on the sawdust-based culture of *L. edodes* vegetative mycerial growth phase on day 30*[1].

Substrate formulations	Ethylene (ng/g fr wt/h)
Sawdust only	0.04
Sawdust+Henneberg*[2]	0.12
Sawdust+Rice bran (4 : 1)	0.35
Sawdust+Wheat bran (4 : 1)	0.38
Sawdust+Corn powder (4 : 1)	0.34
Sawdust+ Rice bran+ Wheat bran+ Corn powder (7 : 1 : 1 : 1)	0.45

*[1] Day 30 : The day just mycelia has fully colonized in the culture.

*[2] Henneberg medium : glucose, 50 g; KNO_3, 2 g; $NH_4H_2PO_4$, 2 g; KH_2PO_4, 1 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; $CaCl_2$, 0.1 g in 1 L of distilled water.

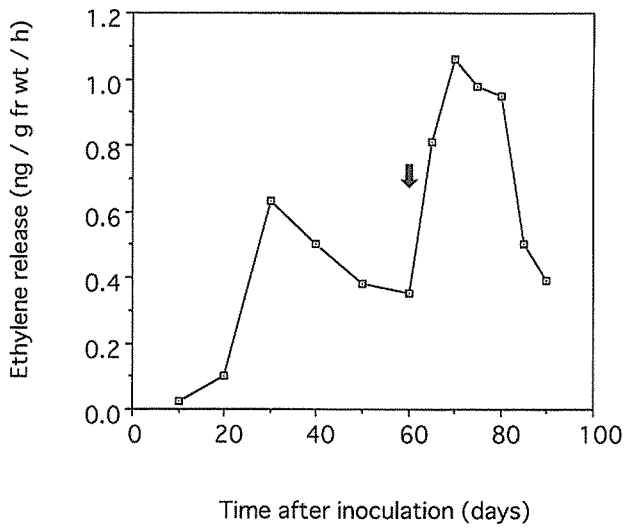

Fig. 3. Production of ethylene in sawdust-based culture during mycelial growth and fruit body development. Mycelial growth phase is from day 0 to day 60, and fruit body development phase is day 60 to day 90. The arrow indicates fruiting treatment on day 60; (the fully colonized mature cultures were transferred from incubation room (20℃) to production room (17℃, 90% RH).

The ethylene is a natural regulator of plant growth ubiquitous among higher plants, and also produced by subcellular particles from rat tissues (Ilag and Curtis, 1968). The ethylene, the simplest unsaturated carbon compound, which is a gas under physiological conditions of temperature and pressure, exerts a major influence on many if not all aspects of plant growth, development, and senescence apparently at regulatory levels of metabolism. The ethylene is regarded as a plant hormone because it is a natural product of metabolism, acts in trace amounts, in conjunction with or antagonistic to other plant hormones, and is neither a substrate or cofactor in reactions associated with major plant development processes. The biochemical origin of ethylene in *Penicillium digitatum* was associated with the TCA cycle and specifically with the middle carbons of the dicarboxylic acids, particularly fumarate. The ethylene derives from the methylene carbons of citrate as they pass through the TCA cycle, because monofluoroacetate, which inhibits conversion of citric acid to isocitric acid, also inhibits ethylene production by *P. digitatum*. It is of interest that glutamic acid, a precursor of ethylene in *P. digitatum* (Chou and Yang, 1973).

It is also suggested that methinine could be a precursor for ethylene in *A. bisporus*. It was proposed that the pattern of ethylene production was a metabolic marker of the expansion stage of *A. bisporus* sporocarp (Turner *et al.*, 1975). A number of volatile hydrocarbons have previously reported to be produced by *A. bisporus* as products both in commercial beds and in axenic laboratory cultures (Turner *et al.*, 1975).

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シイタケ菌床からの二酸化炭素およびエチレンの発生

大 賀 祥 治

要　　約

シイタケ菌床から発生するガスについて，菌糸蔓延から子実体の一次発生までの期間の100日間にわたって経時的に観察した．菌床からは，二酸化炭素とエチレンが発生しており，発生パターンには類似の傾向が認められた．すなわち，菌床内への菌糸蔓延完了時の20－30日目に発生量のピークがみられ，培養60日目に行った低温，高湿の子実体発生操作による子実体誘起に伴って再び発生量が急増し，子実体の開傘時に二回目のピークがみられることが明らかになった．

キーワード：シイタケ，二酸化炭素，エチレン，菌床