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Effect of Some Edible Mushroom Extracts on Fruiting Body Formation of *Volvariella volvacea*

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Abstract

Extracts from some edible mushrooms could supplement important substances for fruiting body formation of straw mushrooms. Crude extracts from young fruiting bodies of *Pleurotus ostreatus*, *Lentinula edodes* and *Agrocybe cylindracea* were diluted and added to potato dextrose agar (PDA) before sterilization. The supplemented agar plates were inoculated with straw mushroom (*Volvariella volvacea*) agar plugs and the growth of the colony was measured after 3 and 7 days. The supplementation with the aqueous extract of *P. ostreatus* resulted in a faster growth and denser mycelia of the straw mushroom than that of the control or the other extract-supplemented media. Twenty ml each of the original solution and the two dilutions (1:1 and 1:3) of the *P. ostreatus* extract was sprayed on the surface of moist cotton waste already fully colonized by straw mushroom mycelia. After 5 days, the dilution of 1:1 significantly stimulated fruiting body formation. This dilution was applied 4 times to the colonized cotton waste substrates before primordial formation. Yield of early mature stage fruiting bodies increased over the control by a percentage of 40. The supplementation with the aqueous extract from *P. ostreatus* could help to induce fruiting bodies and increase the yield of straw mushrooms.

Key words: Cultivation, edible mushrooms, fruiting body, *Pleurotus ostreatus*, *Volvareilla volvacea*.

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Introduction

The straw mushroom (*Volvariella volvacea*) has long been known as a delicious food of nutritious and economic value. It ranked fourth in mushroom production in Thailand (Panuthut 2001). However, its production cannot be increased to industrial scale because of inconsistent yield control. One problem learned from experience is infrequent fruitfulness even when genetic strain selection is carried out and substrate ingredients and physical environments are intensively improved.

The formation of mushroom fruiting bodies is

the most dramatic expression of differentiation and morphogenesis that can be found among the fungi. The principal conditions that have been reported to trigger fruiting body development are mycelial aging, nutrient exhaustion (Niederpruem et al. 1964), mechanical injury (Raper and Krogelb 1958, Leonard and Dick 1973), chemicals (Novak 1996) and natural substances, such as adenosine 3',5'-cyclic monophosphate (cyclic AMP), from fruiting bodies of *Coprinus macrorhizus* (Uno and Ishikawa 1973).

The existence of natural "fruiting-inducing related substances" (FIS) has been proved by the stimulating effect of crude extracts obtained from imperfect fungi (Leonard and Dick 1968) or from higher fungi (Urayama 1969) on the induction of fruiting in higher fungi. Although these substances have demonstrated the same effects across different fungal species, application in edible mushrooms is rare.

The concept of control in fruiting by FIS may be adapted to edible mushrooms. The application of an effective crude extract from an edible mushroom to induce fruiting bodies in a different edible mushroom may be an alternative method of yield control. Furthermore, the application of natural extracts from edible mushrooms will be more acceptable to mushroom consumers and may allow production of edible mushrooms on a commercial scale.

Materials and Methods

Organism and inoculum

The straw mushroom, *Volvariella volvacea*, used in this study was obtained from the Biotechnology Research and Development Office, Department of Agriculture, Thailand. This mushroom had been maintained on a potato dextrose agar (PDA) slant at 25°C. Culture plates prepared in petri dishes (9 mm in diameter) with 20 ml PDA were initially used for inoculation. Agar plugs of 4 mm in diameter taken from the peripheral colony of a given straw mushroom culture on a PDA slant were inoculated on PDA plates. Inoculated plates were kept for 7 days at room temperature.

Selection of effective crude extract in promoting growth of straw mushroom mycelia

Preparation of crude extracts and test plates

Young edible mushrooms, i.e., *Pleurotus ostreatus*, *Lentinula edodes* and *Agrocybe cylindracea* were homogenized separately with distilled water (500 g/l). The aqueous homogenates were centrifuged and their supernatants were obtained. The aqueous extracts were further dried with a lyophilizer. Each lyophilized extract was dissolved in distilled water to make a 1% (w/v) solution, and 20 ml of the solution was added to 80 ml of PDA before sterilization. Five replicates were prepared for each extract.

Measurement of mycelial growth on PDA plate with extract supplement

To test the effect of each crude extract on mycelium growth, a *V. volvacea* agar plug was inoculated onto the center of each test plate. The growth of the mycelia was examined at 30±2°C. The colony diameter on each plate was measured 3 and 7 days after inoculation.

Bioassay of the effective crude extract for straw mushroom growth in cotton waste substrate

The preparation of fermented cotton waste substrate

Small pieces of cotton waste were soaked overnight in water. Then, to 98 g of the cotton waste, 2 g of lime (CAO) were added. The mixture was put into a wooden frame (90 x 90 cm) and piled up to approximately 70-90 cm in height. It was covered with a plastic sheet and placed outdoors for two days for the cotton waste to ferment. Then, it was turned thoroughly by hand. Water was added when needed to maintain moisture content of 70% (150 g dried cotton waste: 350 ml water). The substrate was piled up, covered again, and then further incubated for 2 days (Chang 1988).

Each 500 g of the fermented compost was packed in a polypropylene bag (15 cm x 30 cm), then sterilized at 121°C for 60 min, and left for several hours to cool down to the desired inoculation temperature (30±2°C). Straw mushroom on an agar disk (5 mm in diameter) was inoculated onto the top of the cotton waste substrate in each plastic bag and incubated at 30±2°C for 12 days. Five replicates were prepared.

Application of the effective crude extract and measurement of primordial formation

After the cotton waste substrate was fully colonized with straw mushroom mycelia (12 days) the plastic bag was removed from the lump of substrate. The 1% (w/v) *P. ostreatus* crude extract solution mentioned above was diluted with distilled water to make 0.5% and 0.25% (w/v) solutions. Twenty ml of each solution and of water (control) was sprayed on separate surface of the cotton waste substrate. They were incubated in a plastic container with 80-90% relative humidity at 30±2°C under a light intensity of 1000 lux supplied by fluorescent lamps with a 12 h photoperiod. The cultivation container was ventilated two times a day to keep aeration. Five days after treatment and incubation, the number of primordia (tiny buttons) was counted for each substrate lump.

Measuring the effect of crude extract on straw mushroom yield

Twenty bags of 12 day-old colonized cotton waste (0.5 kg/bag) mentioned above were randomly divided into control and treatment groups. Plastic bags were removed from the lumps of substrate. The colonized cotton waste of the control group was sprayed with 20 ml of distilled water while that of the treatment group was sprayed with the 0.5% (w/v) *P. ostreatus* crude extract solution. All substrate lumps were, then, maintained in the conditions described above. After 10 days, the first harvest of mushrooms was done at an early mature stage. Fresh harvested mushrooms were weighed. Five days after each harvest, the same amount of water or the diluted crude extract solution was sprayed on each of the substrate lumps. Mushroom products were harvested until the fourth flush. The total weight of the harvested mushrooms was considered as yield and was equated to the biological efficiency (the yield of fresh mushroom/ dried weight of the substrate X 100).

Results and Discussion

Young fruiting bodies of edible mushrooms (*Pleurotus ostreatus*, *Lentinula edodes* and *Agrocybe cylindracea*) were processed as described in Materials and Methods to obtain crude extracts. Table 1 shows the amount of crude extracts from each mushroom and percentages of yields.

The crude extracts from these young fresh mushrooms were tested for their bioactivity in increasing straw mushroom growth 3 and 7 days after inoculation. The mean diameter of straw mushroom mycelial growth on *P. ostreatus* aqueous extract agar plate was larger than those on the agar plates of *L. edodes* extract, *A. cylindracea* extract and the control (Table 2).

Table 1 The amount of crude extract and percentages of yield from each mushroom*

Mushroom	Crude extract (g)	Yield (%)
Pleurotus ostreatus	5.04	1.10
Lentinula edodes	8.93	1.78
Agrocybe cylindracea	8.73	1.74

* From 500 g wet weight of fresh mushrooms.

Table 2 Mean diameters of straw mushroom mycelial colony at 3 days and 7 days after inoculation

Type of crude extract	Mean diameter of mycelial colony (mm)	
	3 days after inoculation	7 days after inoculation
Control (DW)	11.9	39.0
P. ostreatus	33.5	54.5
L. edodes	23.5	46.0
A. cylindracea	12.0	43.0

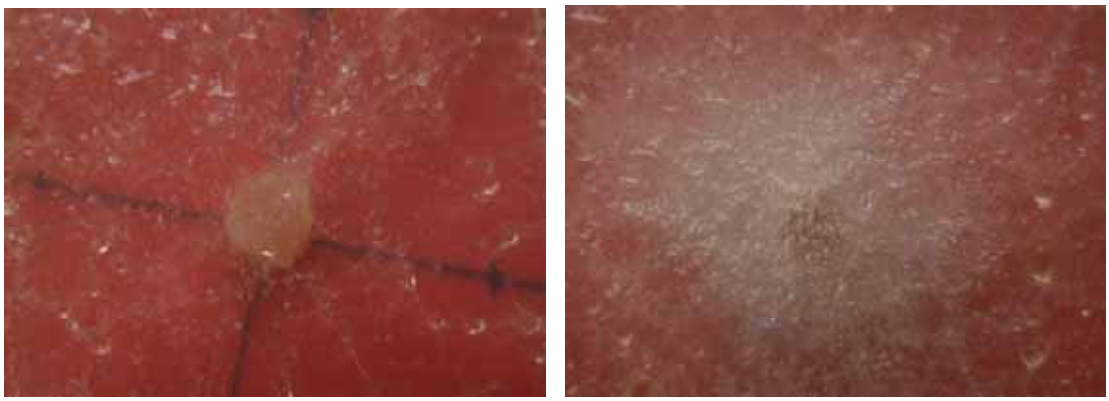

Figure 1 Colonies of straw mushroom observed 7 days after inoculation. Loose colony of straw mushroom observed in a non-supplemented culture (left) and dense colony from a culture with the addition of *P.ostreatus* extract (right).

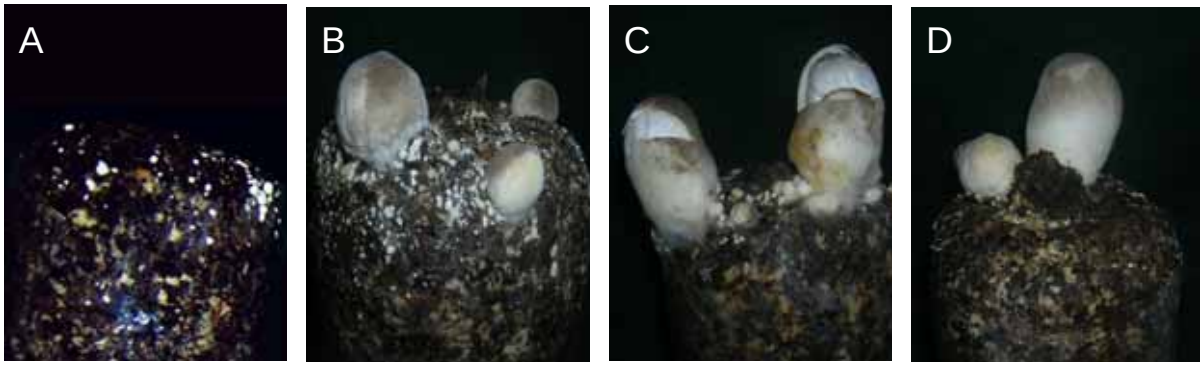

Figure 2 Straw mushroom fruiting bodies growing on cotton waste lumps after supplementation with *P. ostreatus* extract of different concentrations. A: Control (DW), B: Extract diluted 1/4, C: Extract diluted 1/2, D: Extract non-diluted.

Although the difference did not reach a statistically significant level, the supplement with *P. ostreatus* aqueous extract also gave denser mycelial growth compared with other treatments and the control (Table 2 and Figure 1). A prior report indicated that fruiting body formation is correlated directly with mycelial biomass on substrate (Ohga 1992). For this reason, supplement with *P. ostreatus* extract was selected for the study on the induction of straw mushroom fruiting body formation.

The original solution (1%) of *P. ostreatus* extract and its two dilutions (0.5% and 0.25%) were tested for their ability to induce primordial formation of straw mushroom (compared with a control, 20 ml of water). The original solution increased the number of primordia insignificantly compared with the control. The number of primordia in 0.5% solution treatment was significantly higher compared with the treatments with 0.25% solution and non-diluted solution (Table 3 and Figure 2).

Table 3 Effects of different dilutions of *P. ostreatus* crude extract solution on inducing straw mushroom primordial formation

Combination of aqueous extract	Number of primordia[A] ± SE
Water	1.4 ± 0.24^{a}
Aqueous extract diluted 1/4	3.8 ± 0.37^{b}
Aqueous extract diluted 1/2	5.4 ± 0.81^{c}
Aqueous extract	2.0 ± 0.31^{a}

A: The mean of five replicates; SE: Standard error. The treatment means were compared by Duncan's Multiple Rang Test. Values followed by the same letter are not significantly different for $P = 0.05$.

Table 4 Yield of straw mushroom fruit bodies in supplemented and non-supplemented culture on cotton waste lumps

Treatment	Yield of mushroom fruiting bodies (g)					Biological efficiency (%)	Increase over control (%)
	1st flush	2nd flush	3rd flush	4th flush	Total		
Control	149	36	148	63	396[a]	24	0
P. ostreatus extract diluted 1/2	197	90	164	100	551[b]	34	40

a,b: Values with different letters in the same column were significantly different (P=0.038)

The straw mushroom has its highest protein content during the pinhead stage. The high protein content has the potential to result in vigorous growth and differentiation (Chang and Chan 1973). The straw mushroom is relatively inefficient in the utilization of biomass, being unable to consume more complex compounds in the substrate (Chang 2004). On the other hand, *P. ostreatus* is a ligno-cellulolytic mushroom that has the capability to digest various substances. The addition of organic supplements to substrate results in earlier fructification and increases the mushroom yield (Royse *et al.* 1991). The readily prepared bioactive substances from *P. ostreatus* seemed to supplement protein and other nutritional factors for induction of primordial formation of the straw mushroom.

The colonized cotton waste lumps (0.5 kg/bag) were sprayed with either distilled water or 0.5% crude extract solution. Mushroom fruiting bodies were harvested four times. The yield in each flush in the extract-supplemented group was higher than that of the non-supplemented group (Table 4). This led to higher total product weight in the treated group than in the control group. In the first flush from the extract-supplemented group, the mushroom yield was higher than in the following flushes. Totally, the addition of *P. ostreatus* extract could increase the biological efficiency of the straw mushroom over that of the control by a level of 40% (Table 4). From Figure 3, the profile of cumulative yield in the extract-supplemented group also is steeper than in the non-supplemented group.

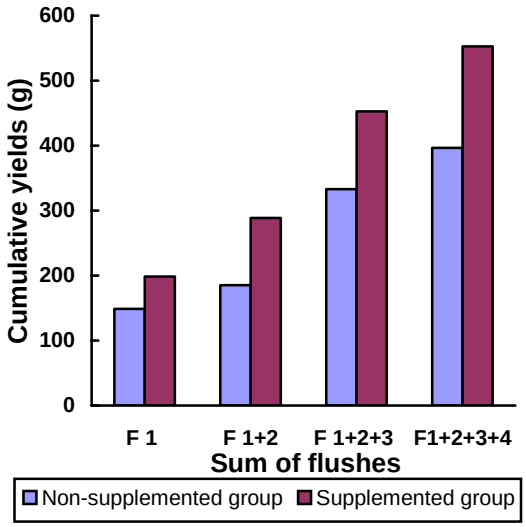

Figure 3 Cumulative yields of each flush in supplemented and non-supplemented groups

During the development of the straw mushroom fruiting body, the requirement for protein is higher again in the elongation stage especially in pileus, volva and stipe (Chang and Chan 1973). Although cotton waste substrate has a protein content with a percentage of 10.63± 0.05 (Silva *et al.* 2002), the insufficiency of digestive enzymes in the straw mushroom might limit the utilization of proteins in the substrate. Supplementation of *P. ostreatus* extract could help to increase straw mushroom growth directly and indirectly. The *P. ostreatus* extract has a higher percentage of protein content (20.03 ± 0.04) (Silva *et al.* 2002) than that of cotton waste. It can fulfill the protein requirement during rapid growth of fruiting bodies. Indirectly, the extract contains bioactive enzymes that could digest the substrate to produce amino acid

molecules and nutritional factors essential for straw mushroom growth. When the number of fruiting bodies and the weight of each fruiting body were increased, total weight and product yield could be raised.

Furthermore, from the economic aspect, the oyster mushroom (*P. ostreatus*), as a source of effective extract, can be cultivated successfully over a wide range of geographical locations in natural conditions without any need for artificial temperature or humidity control (Basak *et al*. 1996). Therefore, the abundant availability of *P. ostreatus* allows us to produce it easily and make the cost low.

Conclusion

Aqueous extract of *P. ostreatus* contains ready available bioactive substances that can stimulate the growth and development of the straw mushroom. Furthermore, the extract can help to improve the primordial formation and product yield in straw mushroom cultivation. To fractionate bioactive components in the aqueous extract and to carry out a bioassay to test their activities is necessary.

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