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# Growth promoting effects of water extract of *Armillaria mellea* rhizomorph on mycelia of *Polyporus umbellatus*

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**Abstract** – The authors investigate the effects of different concentrations of water extract of *Armillaria mellea* rhizomorphs on mycelial growth of *Polyporus umbellatus*. This study shows that water extract of *A. mellea* rhizomorph served as a good carbon and nitrogen source for mycelial growth of *P. umbellatus*, with 6‰ concentration of water extract of *A. mellea* rhizomorph exhibiting the best growth-promoting results.

**Polyporaceae / Water extract / Physalacriaceae / Growth-promoting effects**

## INTRODUCTION

*Polyporus umbellatus* (Pers.) Fr., a medicinal fungus belonging to Polyporaceae, Basidiomycetes, has been widely used in China for a long time (Xing & Guo, 2005; Xing *et al.,* 2011; Zhou & Guo, 2009). Its sclerotia have profound health promoting benefits used in treatment of edema, acute nephritis, diarrhea and promotion of diuresis (Huang & Liu, 2007; Liu and Guo, 2010; Zhang *et al.,* 2010). It has been reported that *P. umbellatus* possesses outstanding anti-tumor properties as well (Miyazaki *et al.,* 1978; Ohsawa *et al.,* 1992; You *et al.,* 1994; Mohammad *et al.,* 2007), whereas an ergostane derivative isolated from *P. umbellatus* could inhibit rabbit platelet aggregation (Zjawiony, 2004). Sekiya *et al.* (2005) reported inhibitory effects of *P. umbellatus* on free radical-induced lysis of red blood cells, while Yi & Ken (2008) suggested that ecdysteroids extracted from the sclerotium of *P. umbellatus* had remarkable anti-inflammatory effects on ear edema in mice.

However, *P. umbellatus* is in danger of extinction in China due to ineffective protection from serious habitat destruction and unrestricted harvesting (Huang & Liu, 2007) and because its mycelial growth is much slower than that of many other mushroom species, it is difficult to cultivate *P. umbellatus* on a large scale (Huang & Liu, 2007). Therefore, finding ways to shorten the growth period of *P. umbellatus* can be part of the solution for the mass production of *P. umbellatus* under artificial conditions.

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*Armillaria mellea*, a serious root rot disease causing fungus in family Physalacriaceae (Rizzo *et al.* 1998, Ng *et al.,* 2007; Lung & Huang, 2010) is another popular medicinal mushroom in China (Kim *et al.,* 2008). It has been well documented that this mushroom is capable of treating headache, palsy, insomnia and microbial infectious diseases (Wu *et al.* 2007). Water-soluble components and polysaccharides are suggested to be especially important for its immune-modulating activities and anti-tumor effects (Mohammad *et al.,* 2007; Wu *et al.,* 2007; Kim *et al.,* 2008).

In nature, sclerotial development of *P. umbellatus* seems related to the presence of rhizomorphs of *A. mellea* (Choi *et al.,* 2003). The rhizomorph of *A. mellea* adheres and invades sclerotia of *P. umbellatus* (Guo & Xu, 1993; Choi *et al.,* 2003) and subsequently absorbs nutrients from the mycelium of *P. umbellatus* (Gu *et al.,* 2008). At the same time, part of the fungal mass of *A. mellea* rhizomorph is digested by sclerotia of *P. umbellatus* as a nutrient source (Guo & Xu, 1992; Choi *et al.,* 2003). Thus, it was suggested that *A. mellea* formed a symbiosis with *P. umbellatus* (Guo & Xu, 1992; Guo & Xu, 1993) and the orchid *Gastrodia elata* (Lung & Huang, 2010). Xu *et al.* (2003) suggested that sclerotial growth of *P. umbellatus* was only possible in symbiosis with *A. mellea*. Recently, Kikuchi & Yamaji (2010) reported that apart from *A. mellea*, other *Armillaria* species such as *A. sinapina*, *A. calvescens, A. gallica, A. cepistipes,* and *A. nabsnona* also had a relationship with the sclerotia of *P. umbellatus*.

Xu & Guo (1992) first demonstrated that mycelial growth of *P. umbellatus* could be accelerated by adding grounded powder of *A. mellea* rhizomorphs in solid-state cultivation. Later, it was reported that the metabolites of *A. mellea* provided nutrients for sclerotial of *P. umbellatus*. (Wang *et al.,* 2000) and that the symbiosis of *A. mellea* and *P. umbellatus* resulted in generation of active oxygen (Xia & Guo, 2000; Xia & Guo, 2001). An oxidative burst was called a rapid rise in reactive oxygen species level (Cho *et al.,* 2009). Recent studies also showed that after *A. mellea* rhizomorphs invade the mycelia of *P. umbellatus* in dual cultures, *A. mellea* almost stops growing because of a strong inhibition exerted by *P. umbellatus* (Xing & Guo, 2003) which makes it difficult to obtain growth-promoting substances from the living *A. mellea* rhizomorphs. Which active constituents in *A. mellea* are responsible for promoting the mycelial growth of *P. umbellatus* is still unclear. In this study, the authors explore for the first time growth-promoting effects of water extract of *A. mellea* on *P. umbellatus* as measured through colony diam. and growing rate. We hope that our findings may contribute to further research concerning the potential use of active ingredients of *A. mellea* as inoculants to promote growth of *P. umbellatus* during commercial production.

## MATERIALS AND METHODS

*P. umbellatus* used in this study was collected and isolated by Shun-Xing Guo in Guxian, Shanxi Province of China. *P. umbellatus* was maintained on wheat bran agar slant medium (wheat bran 3%, glucose 2%, $KH_2PO_4$ 0.046% $MgSO_4$ 0.05%, agar 1%) at 4°C. Dry rhizomorph of *A. mellea* was collected from Shanxi Province of China.

Complete medium (positive control) was composed of peptone 0.2%, glucose 2%, $MgSO_4$ 0.05%, $KH_2PO_4$ 0.046%, $K_2HPO_4$ 0.1%, vitamin B1 50ug/L

and agar 1%. Basic medium (negative control) contained all substances in complete medium except peptone and glucose. Both the complete and basic medium were autoclaved at 121°C for 20 min. Vitamin B1 was sterilized by filtration through a 0.22-μm Millipore filter and then added to the cooled autoclaved medium. One hundred grams of dry rhizomorph of *A. mellea* was added to water three times and left in it for three hours each time and then this water extract was filtered and finally condensed to obtain 3 grams of equivalent extract (Guo & Guo, 2008). Different quantities of water extract of *A. mellea* rhizomorph (0.3 g, 0.6 g and 0.9 g per liter) were autoclaved and added to the basic medium to make three different mediums named W(l), W(m) and W(h) with respective concentrations of 0.3‰(l), 0.6‰(m) and 0.9‰(h). The five mediums (complete medium, basic medium, W(l), W(m) and W(h)) as mentioned above were used to culture *P. umbellatus.*

The inoculums of *P. umbellatus* were obtained from wheat bran agar slant culture after incubation in malt extract agar medium (malt extract 1%, glucose 2%, peptone 0.6% and agar 1%) at 25°C without light for 20 days and then activated twice. After that, mycelial plugs of 6 mm diam. from actively growing colonies were transferred to the center of 9 cm diam. petri dishes containing 20 ml solid agar medium, at 25°C, under dark conditions, with five repeats for each group. Then mycelial growth of *P. umbellatus* was estimated by measuring colony diameters at 21 days, 28 days and 35 days after inoculation. All experiments were repeated three times. All values represent the mean of five independent measures. The difference of colony diameters of *P. umbellatus* between groups was analyzed with One-way ANOVA, using SPSS 11.0 (SPSS Inc., Chicago, IL, USA).

## RESULTS

Our results show that three different concentrations of a water extract of rhizomorphs of *A. mellea* can significantly increase the growth of *P. umbellatus* compared to that on complete medium alone ($P<0.05$). There was no significant difference between W(l), W(m) and W(h) groupsm although *P. umbellatus* grew the fastest in W(m) group. It did not grow in the negative control group (Table 1). After *P. umbellatus* was cultured for 35 days, it no longer grew in basic medium. Aerial hyphae of *P. umbellatus* grew more exuberantly in W(l), W(m) and W(h) groups than in the complete medium (Fig. 1).

## DISCUSSION

Due to the slow growth of mycelium of *P. umbellatus* in culture, it is important that the long lag phase during cultivation can be shortened. Although solving this problem needs additional research, we presented here evidence for the stimulating effect of water extract of *A. mellea* rhizomorphs on mycelial growth of *P. umbellatus* expressed as hyphal diam. extension and growth rate. Our study shows now that a concentration of 6‰ water extract of *A. mellea* rhizo-

Table 1. Growth promoting effects of water extract of *A. mellea* on *P. umbellatus* mycelia in different incubation periods

| *Different group* | *Diameter of colonies of* P. umbellatus | | |
|---|---|---|---|
| | *21 days* | *28 days* | *35 days* |
| Negative control | 0 | 0 | 0 |
| Positive control | 2.26 ± 0.07a | 2.60 ± 0.03a | 2.67 ± 0.09a |
| W(l) | 2.48 ± 0.04b | 3.01 ± 0.07b | 3.08 ± 0.08b |
| W(m) | 2.53 ± 0.08b | 3.10 ± 0.07b | 3.12 ± 0.06b |
| W(h) | 2.47 ± 0.03b | 3.04 ± 0.04b | 3.10 ± 0.03b |

Values are presented as the mean ± SE.
Values designated with different letters are significantly different at $P < 0.05$.

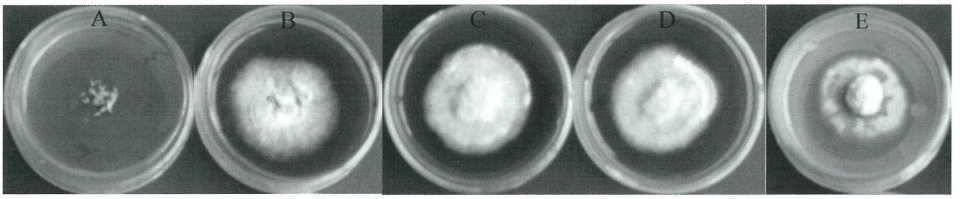


Fig. 1. Colony morphology of *P. umbellatus* in different mediums after 35 days' incubation (A) negative control group (B), (C) and (D) respectively represented for W(l), W(m) and W(h) group (E) positive control group.

morph could significantly accelerate growth of *P. umbellatus* in solid-state cultures compared to *P. umbellatus* in the traditional medium containing peptone and glucose. These findings can be of great practical significance.

Which of the different constituents in the water extract of *A. mellea* stimulates growth of *P. umbellatus*, and whether other extracts of *A. mellea* can have positive or negative effects on the growth of *P. umbellatus* is still under research. *A. mellea* was found to be a good source of carbohydrates, proteins, and trace minerals (Paraskevi *et al.,* 2009), and our water extract of *A. mellea* rhizomorphs might contain water-soluble components or polysaccharide that could be responsible for the growth-promoting effects in providing necessary nutrients or a better concentration of carbon/nitrogen for mycelia of *P. umbellatus*. Another explanation is that the involvement of growth-stimulating substances such as hormones in *A. mellea* renders the growth-promoting results (Zhang *et al.,* 1999).

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