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Growth Conditions and Antifungal Activities of *Oudemansiella mucida*

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Abstract: *Oudemansiella mucida* is an edible mushroom belongs to Homobasidiomycetes. The growth conditions and antifungal activities of *O. mucida* were evaluated. This mushroom was showed an exceptional response to pH. The mycelial growth and weight was increased with the increase and decrease of pH value. The acidic pH (4~6) value was better for its mycelial growth. Temperature suitable for mycelial growth and weight was obtained at 20~25°C and no mycelial growth was recorded at 35°C. The liquid culture filtrate and solid culture extract (water and ethanol) were used against *Botrytis cinerea*, *Colletotrichum gloeosporioides* and *Colletotrichum miyabeanus*. The lowest and similar inhibition of *B. cinerea* was found in each condition of 3 extracts. In liquid culture filtrate and water extract, inhibition of *C. gloeosporioides* was better than *C. miyabeanus*. But in ethanol extract, inhibition of *C. miyabeanus* was found to be better than *C. gloeosporioides*. Water extract of solid culture was showed the best inhibitory effect against the growth of plant pathogenic fungi.

Key words: Antifungal activities・crude extracts・growth condition・PIMG

INTRODUCTION

Oudemansiella mucida is an edible mushroom belongs to Homobasidiomycetes, Tricholomataceae. The genus *Oudemansiella* is saprophytic and found mostly growing on wood. They tend to produce large quantities of white spores. Beside *O. mucida*, several species of this fungus such as *O. radicata*, *O. canarii*, *O. orientalis*, *O. hongoi* and *O. tanzanica* are consumed worldwide. According to culinary status, 24 edible mushrooms species are recorded in Nepal and *O. radicata* is one of the best having good taste [1]. This fungus has long back ground of ethno-botanical use against fungal pathogen. To the class of rare medicinal mushrooms in Siberia, the *O. mucida* is a recognized species [2]. *O. canarii* showed strong inhibition in the bioautographic assay with *Cladosporium sphaerospermum* and chromatographic fractionation guided by this assay allowed the isolation of oudemansin, a known fungitoxic compound [3]. The *O. radicata* is known to produce several bioactive compounds denominated strobilurins and oudemansins. They are able to inhibit fungal growth at very low concentrations without any significant antibacterial activity [4-6]. These compounds kill opportunist pathogens such as *Candida albicans* and dermatophytes belonging to the genus *Trichophyton*, *Epidermophyton* and *Microsporum* [7]. The culture extract of *O. canarii* presented a wide antifungal spectrum, inhibiting the growth of *C. albicans*, *C. glabrata*, *C. krusei* and *C. tropicalis*. It is reasonable to expect that the observed activity be due to the presence of one more compound [8]. This is the first report on the antifungal activity of this species.

In our previous research, we studied the antimicrobial effects of 9 Korean wild mushrooms and indicated that *O. mucida* has good antimicrobial activities [9]. In this research we extended our study and presented in this paper.

MATERIALS AND METHODS

Used microorganisms: *Oudemansiella mucida* IUM929 was obtained from "Culture Collection of Wild Mushroom Species (CCWM)", University of Incheon and used in this experiment. The mushroom strain was maintained on potato dextrose agar (PDA) medium at 25°C for further study. Three plant pathogenic fungi include *Botrytis cinerea*, *Colletotrichum gloeosporioides* and *Colletotrichum miyabeanus* were obtained from "Center for Fungal Genetic Resources (CFGR)" and used for this study. The strains of plant pathogenic fungi were maintained on PDA at 25°C.

Effect of temperature: Six different temperatures were used to screen the suitable mycelial growth and weight of *O. mucida*. For mycelial growth, a 5 mm diameter plug

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removed from 10 days old culture grown on PDA was placed in the centre of each plate filled with PDA. The inoculated Petri plates were then incubated at 10, 15, 20, 25, 30 and 35°C for 10 days separately. The measurement of mycelial growth was performed according to the method described by Shim *et al.* [10]. To determine the mycelial weight, 100 ml potato dextrose broth (PDB) in 250 ml of conical flask was inoculated with 5 mm diameter agar plug removed from 10 days old culture of *O. mucida* grown on PDA and incubated at 10~35°C, on rotary shaker 140~150 rpm for 10 days separately. After incubation, the entire fungal mycelium was harvested by filtering through previously dried and weighted Whatman filter paper No 1. It was then dried to constant weight at 65°C. Before weighting, the filter paper were allowed to cool and subsequently weighted in a balance. The weight of the mycelium was calculated by deducting the weight of filter paper from the final weight. All pairings were carried out in 10 replicates.

Effect of pH: To screen pH suitable for the mycelial growth and weight pH 4~9 was observed. PDA and PDB medium was used to study the mycelial growth and weight, respectively. The both medium was adjusted to the range of pH 4~9 separately with the addition of 1 N NaOH or HCl. For mycelial growth, a 5 mm diameter plug removed from 10 days old culture grown on PDA was placed in the centre of each plate filled with PDA. The inoculated Petri plates were then incubated at 25°C for 10 days. The measurement of mycelial growth was performed according to the method described by Shim *et al.* [10]. In case of mycelial weight, 100 ml PDB in 250 ml of conical flask was inoculated with 5 mm diameter agar plug of *O. mucida* and incubated at 25°C, on rotary shaker 140~150 rpm for 10 days separately. After incubation, the entire fungal mycelium was harvested, weighted and calculated similarly. All pairings were carried out in 10 replicates.

Collection of crude extract: The fungus *O. mucida* was cultured both in potato dextrose broth (PDB) and PDA medium separately. PDB culture was incubated at 25°C, on rotary shaker 140~150 rpm and PDA culture was incubated at 25°C for 30 days. After incubation, solid culture was dried in fume hood (HK-FH1800, Korea), powdered and then extracted both in distilled water and 70% ethyl alcohol (1 g: 15 ml) separately for 72 hours at 25°C. To obtain filtrates, the liquid culture, water extract and ethanol extract were filtered through 2 layers of Whatman No. 1 filter paper. The 3 filtrates were concentrated by a rotary evaporator (Eyela, Tokyo Rikakikai Co. Ltd., Japan) until semi-solid state substances were obtained. The semi-solid state substances were then freezing dried at-80°C (Operon, Korea). Thus the samples were ready for further use.

Assay of antifungal activity: For quantitative assay, the method percent inhibition of mycelial growth (PIMG) was used to determine the antifungal effect of 3 crude extracts of *O. mucida*. The crude extracts of liquid culture filtrate, water extract and ethanol extract were added to PDA at the concentrations of 10, 20 and 40 mg ml^{-1} separately. After autoclaving at 121°C for 15 minutes, these were poured in 55 mm sterilized Petri dishes. Agar discs (5 mm) were taken from 10 days old cultures of 3 plant pathogenic fungi and placed in the center of the Petri plates separately. For control, same size agar discs of 3 fungi were placed in a same way on a fresh PDA plate. All pairings of cultures were carried out in 5 replicates and incubated at 25°C for 6 days. Inhibitory activity was assessed by measuring the radial growth of mycelium on treated culture (R_2) and the radial growth on fresh PDA as control plate (R_1). The two measurements were transformed in to PIMG using the formula [11], where $PIMG = \{(R_1-R_2)/R_1\} \times 100$.

For qualitative assay, the antifungal activity was carried out in 87 mm Petri plates containing 20~25 ml PDA. Agar discs 5 mm were taken from 10 days old cultures of 3 plant pathogenic fungi and placed in the center of the Petri plates separately. After the mycelial colony had developed, sterile blank filter paper discs at a distance were placed 1 cm away from the rim of the mycelial colony. The samples were dissolved in sterilized distilled water at 3 concentrations of 25, 50 and 100 mg ml^{-1}. Then 50 μl of aliquot from separate concentration was applied to discs separately. The plates were incubated at 25°C until mycelial growth had enveloped peripheral disks containing the control and had formed crescents of inhibition around the paper discs containing samples with antifungal activity.

RESULTS AND DISCUSSIONS

Effect of pH: To screen pH value suitable for a favorable mycelial growth and weight, the range of pH 4~9 was observed. *O. mucida* showed an exceptional response to pH. The mycelial growth and weight was increased with the increase and decrease of pH value. The acidic

Table 1: Effect of pH and temperatures on the mycelial growth and weight of *O. mucida*

	pH effect			Temperature effect	
pH	Mycelial growth	Mycelial weight	°C	Mycelial growth	Mycelial weight
4	64±4.17	0.76±0.03	10	22±3.00	0.25±0.04
5	66±3.04	0.64±0.02	15	37±2.20	0.41±0.03
6	61±3.61	0.60±0.03	20	69±3.47	0.77±0.03
7	48±3.17	0.47±0.02	25	78±3.81	0.82±0.02
8	51±2.15	0.57±0.02	30	37±6.08	0.60±0.05
9	59±4.25	0.60±0.04	35	-	-

Mycelial growth (mm) and mycelial weight (g) was measured (n=10) after 10 days of incubation

Table 2: Inhibitory effect of different crude extracts on the mycelial growth of 3 plant pathogenic fungi

	PIMG								
	Liquid culture filtrate			Water extract			Ethanol extract		
Plant pathogenic fungi	A	B	C	A	B	C	A	B	C
B. cinerea	11.79	16.29	22.59	11.56	15.75	21.37	9.93	17.21	27.14
C. gloeosporioides	38.18	49.39	59.15	69.77	76.13	81.25	42.18	52.36	71.10
C. miyabeanus	38.37	44.63	54.36	67.91	71.20	73.21	64.66	70.66	77.34

Percent Inhibition of Mycelial Growth (PIMG) was calculated (n = 5) after 6 days of incubation at 25°C. A, B and C contains 10, 20 and 40 mg ml^{-1} respective extract, respectively

pH (4~6) value was better for the growth of this fungus. The lowest and highest mycelial growth was at pH 7 and 5, respectively. In case of mycelial weight, the lowest and highest was at pH 7 and 4, respectively. The pH 6 was better than 9 (Table 1). The pH 7 is the most suitable for the optimal growth of *Macrolepiota procera* and our results oppose to this finding [12]. Mycelial growth of *Phellinus japonica* and *Phellinus linteus* was optimal at pH 7 and 6~7 respectively [13, 14]. The optimal pH of *Paecilomyces sinclairii was* 8 [15]. Shim *et al.* [10] also reported that the most favorable and most unfavorable pH of *Grifola umbellata* was 4 and 9 respectively which is fully similar to our finding. This result suggested that mushrooms may have a broad pH range for their optimal mycelial growth in nature.

Effect of temperature: Two parameters the mycelial growth and weight were observed in this study. Temperature suitable for mycelial growth and weight was obtained at 20~25 and no mycelial growth was recorded at 35°C. The lowest growth and weight was found at 10°C. The effect of 15 and 30°C on mycelial growth was similar but in case of mycelial weight 30°C was better than 15°C (Table 1). The mycelial growth of *P. fumosoroseus* had been expedited gradually in proportion to the rise of temperature and was the most suitable at 25°C [15, 16]. Even though the mycelial growth of *P. fumosoroseus* was favorable at the range of 20 to 25°C and had been expedited in proportion to the rise of temperature, the mycelial growth appeared to be suppressed at the temperature higher than 30°C. The favorable mycelial growth of *M. procera* and *Pleurotus ostreatus* was at 30°C [12, 17]. So, this discussion is similar to present result.

Antifungal assay: Three crude extracts such as liquid culture filtrate, water extract and ethanol extract of *O. mucida* were used against mycelial growth of *B. cinerea, C. gloeosporioides* and *C. miyabeanus* phytopathogenic fungi (Table 2 and Fig. 3 and 4). The lowest and similar inhibition of *B. cinerea* was found in each condition of 3 extracts. In liquid culture filtrate and water extract, inhibition of *C. gloeosporioides* was better than *C. miyabeanus*. But in ethanol extract, inhibition of *C. miyabeanus* was found to be better than *C. gloeosporioides*. Among 3 samples, water extract was the best on the inhibition of mycelial growth of 3 plant pathogenic fungi. For qualitative data, we studied secondary metabolite, water and ethanol extract against mycelial growth of plant pathogenic fungi. All of the tested samples were showed good antifungal effect (Fig. 5). The inhibitory effect of 9 mushrooms and

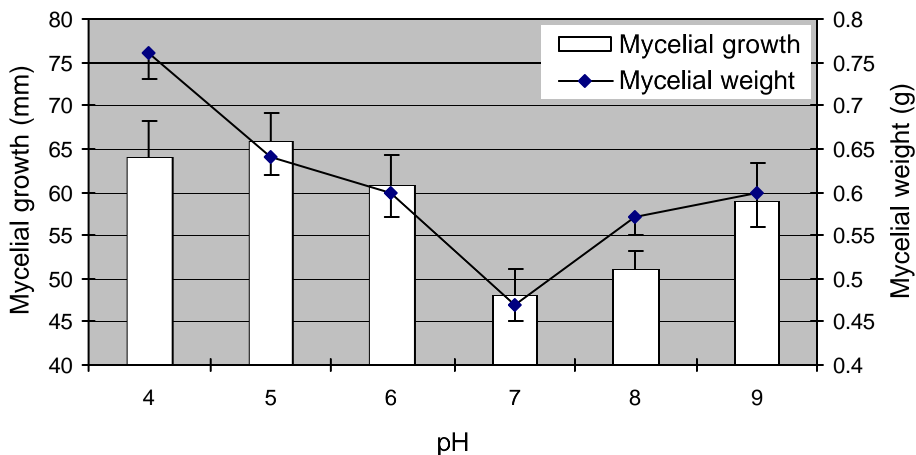

Fig. 1: Effect of pH on the mycelial growth and weight of *O. mucida*. Mycelial growth (mm) and mycelial weight (g) was measured (n = 10) after 10 days of incubation

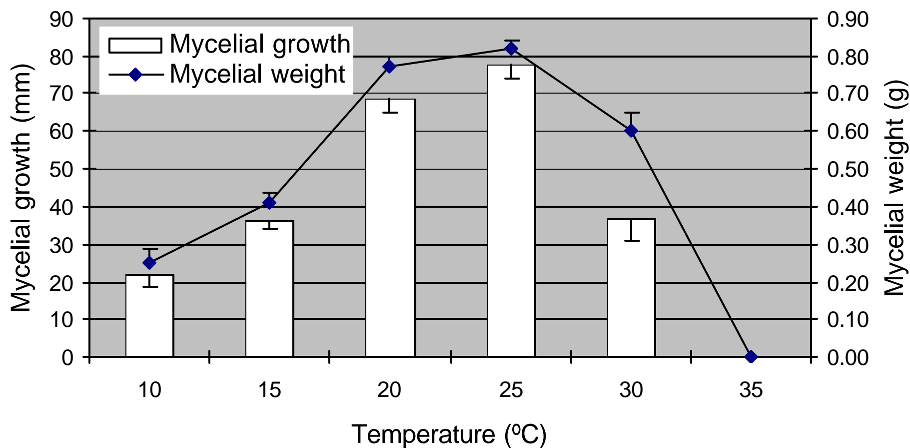

Fig. 2: Effect of temperatures on the mycelial growth and weight of *O. mucida*. Mycelial growth (mm) and mycelial weight (g) was measured (n = 10) after 10 days of incubation

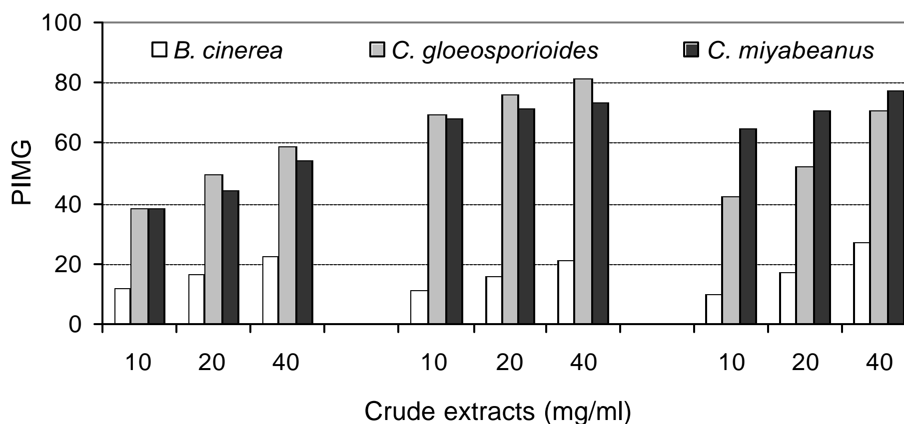

Fig. 3: Inhibitory effect of liquid culture filtrate (left), water extract (middle) and ethanol extract (right) on the mycelial growth of 3 plant pathogenic fungi. Percent Inhibition of Mycelial Growth (PIMG) was measured (n = 5) after 6 days of incubation at 25°C

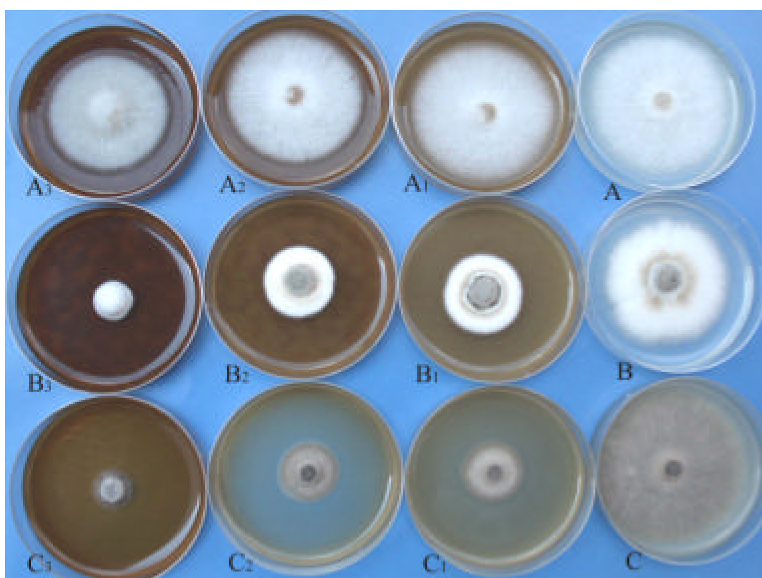

Fig. 4: Inhibitory effects of ethanol extract on the mycelial growth of *B. cinerea* (A), *C. gloeosporioides* (B) and *C. miyabeanus* (C). A, B and C indicates control (fresh PDA) of respective fungi. $A_1/B_1/C_1$, $A_2/B_2/C_2$ and $A_3/B_3/C_3$ contain 10, 20 and 40 mg ml^{-1} crude extract, respectively. Percent Inhibition of Mycelial Growth (PIMG) was calculated (n = 5) after 6 days of incubation at 25°C

Fig. 5: Inhibitory effect of secondary metabolite and crude extracts on *C. miyabeanus.* A, B and C contain 25, 50 and 100 mg ml^{-1} concentrated solution, respectively. W: Distilled water, F: Benomyl 1000 ppm (upper). A: Water extract (50 mg ml^{-1}), B: Ethanol extract (50 mg ml^{-1}), H_2O: Distilled water, F: Benomyl 2000 ppm (down). Each filter paper disc contains 50 µl of aliquot

found good result to inhibit the mycelial growth and weight of plant pathogenic fungi [9]. The antifungal peptide collected from *Pleurotus ostreatus* showed good inhibitory effect against *Fusaerium oxysporum, Mycosphaerella arachidicola* and *Physalospora piricola*. They also added Pleurostin to PDA at 3 doses and examined the growth phenotype of the pathogenic fungi [18]. Jonathan and Fasidi [19] studied the antifungal effect of two *Lycoperdon* spp. and the best antifungal activity was recorded in *Lycoperdon giganteum* ethanol extract against *Microsporum boulardil*. They focused that the higher fungus is a promising antifungal agent but the observed values for all other extracts against pathogenic fungi were low. Lam and Ng [20] designed *Lyophyllum* antifungal protein (LAP) isolated from mushroom (*L. shimeji*) which exerted antifungal activity against *P. piricola* and *M. arachidicola* but not against *Rhizoctonia solani, Colletotrichum gossypii* and *Coprinus comatus.* They reported antifungal effect of *Lentinus edodes* and identified the compound as straight-chain alcohol with 8-9 carbons having double and triple bonds was active on filamentous fungi [21].

CONCLUSIONS

This study exposed environmental culture conditions and antifungal effects of different crude extracts of *O. mucida* were used *in vitro*. Therefore, it could be suggested that, the obtained results could be useful for mass cultivation and evaluating compounds of interest produced by this fungus.

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