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Effect of Various Sawdusts and Logs Media on the Fruiting Body Formation of *Phellinus gilvus*

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Present experiments were conducted to determine the possibility of artificial culture with various sawdust of *P. gilvus*. The pH value was 6.0 of oak sawdust, 6.5 of mulberry sawdust, 6.6 of elm sawdust, 6.3 of acacia sawdust and 6.1 of apple tree sawdust. Mycelial density on elm sawdust and acacia sawdust were lower than those of oak sawdust, and apple sawdust. Weight of fresh fruiting body showed that 179 g on oak tree, 227 g on oak sawdust, 21 g on elm tree, 76 g on elm sawdust, 106 g on apple tree, and 170 g on apple sawdust. Among them, the yield of oak substrates was the highest whereas acacia sawdust was the lowest, and it is concluded that the yields of sawdust substrates were higher than log substrates. *P. gilvus* grown on various sawdusts and logs used in this study have shown similar in anti-tumor activity against P388.

KEYWORDS: Antitumor activity, *Phellinus gilvus*, Sawdust culture

Genus *Phellinus* is taxonomically classified into Aphyllophorales in Hymenochaetaceae of Basidiomycota (Larsen and Cobb-Poulle, 1990) and has been also known as a plant pathogen that causes white pocket rot and severe plant diseases such as canker or heart rot in living trees (Gilbertson, 1980). *Phellinus* spp. is known about 220 species and is found mainly in tropical America, Africa (Dai and Xu, 1998). In Korea, it is distributed into 6~8 species and commonly referred to as Sangwhang (Lee, 1993; Hong, 2000). Recently, many reports demonstrated that *Phellinus* species contained medicinally valuable substances. In Asian countries such as China, Korea and Japan, *Phellinus* species have been considered to cure stomachache and arthritis as an oriental medicine (Ying *et al.*, 1987). It was reported that polysaccharide from *P. linteus* showed immuno stimulating activity (Lee *et al.*, 1996), an inhibitory effect on tumor growth and metastasis (Han *et al.*, 1999) and water extracts of *P. linteus*, *P. baumii*, and *P. gilvus* have shown anti-tumor activity against both sarcoma 180 and P388 (Bae *et al.*, 2004), *P. gilvus* TMC-1117 showed biphasic vasodilator activity on rat aorta with endothelium (Hosoe *et al.*, 2006). The physiological characteristics, chemical composition and cultivating methods of *P. linteus* including other species of *Phellinus* have been intensively researched (Chi *et al.*, 1996; Jung *et al.*, 1997 and Rew *et al.*, 2000). *Phellinus* species have been isolated from various geographical regions and it was reported that *P. linteus* occurs mainly on *Morus* and various species of *Quercus* in Korea (Kim *et al.*, 1999) and *P. gilvus* causes a white-pocket rot in *Eucalypus crebra* F. Muell, *Eucalyptus diversicolor* F. Muell (Barry *et al.*, 2005) and occurs mainly broadleaf trees (Lee, 1993). Despite such a great medicinal value, study on artificial cultivation of *P. gilvus* has been rarely conducted. This present study was especially tried to elucidate the possibility of the artificial production of *P. gilvus* by cultural method using various sawdusts and logs.

Materials and Methods

Substrates analysis. Chemical compositions of substrates analysis applied correspondingly to RDA Soil Physico-Chemistry Analysis (Han, 1988). CaO, MgO and K_2O analyzed with Atomic Absorption Spectrometer (Perkin Elmer 2380). Carbohydrate, nitrogen, P_2O_5 and pH analyzed with Tyurin assay, Kjeldahl assay, Colorimetric assay and pH-Meter (Fisher model 50), respectively.

Fungal isolates. *Phellinus gilvus* (KCTC 6653) was obtained from Biological Resource Center, KRIBB., and cultured at 30°C on potato dextrose agar (PDA) medium. The medium was consisted of 0.4% potato extract, 2% dextrose, 1.5% agar and adjusted volume to 1,000 *ml*. Subcultures were made routinely every 10 days.

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Inoculation. The PDA medium was sterilized for 20 minutes at 121°C and aseptically poured into a petri-dish. After cooling, a piece of mycelia was inoculated on the PDA medium plate to use as an inoculum for the next step.

Mother spawn. The sawdust of oak tree (*Quercus* spp.) was mixed with rice bran at a ratio of 9 : 1 (v/v) and moisture content was adjusted to about 65% by adding water. Then the mixed medium was put into a 250 *ml* flask and sterilized at 121°C for 90 minutes. After cooling to 20°C, a piece of mycelia from the PDA plate was inoculated on the sawdust medium to use as inoculum for the planting spawn.

Planting spawn. The planting spawn medium was prepared by the same method as used for the mother spawn. The medium containing sawdust and rice bran put into 850 *ml* polyethylene bottle and sterilized at 121°C for 90 minutes and cooled at 20°C, mother spawn was inoculated to the sawdust culture medium in 850 *ml* polyethylene bottle. The inoculated sawdust media were incubated at 25°C for about 35 days until mycelia spreaded all over the media and then used an inoculum for cultivation.

Sawdust cultivation process. The cultivation method for *P. gilvus* was modified orderly as follows; substrate preparation, transfer substrates to polyethylene bag, sterilization, inoculation, spawn run, initiation of primordium, and growing of basidiocarps.

Substrates and preparation. A variety of sawdusts collected from a local sawmill in Andong City, Gyeongsangbuk-Do, Province. The sawdust was collected and was stored in an enclosed warehouse until it was used. The media were prepared with sawdust of *Quercus acutissima* (oak), *Morus alba* (mulberry), *Ulmus davidiana* (elm), *Malus pumila* (apple) and *Robinia pseudoacacia* (acacia). The moisture of each sawdust medium adjusted to about 65% with water. 2 kg each medium put into a polypropylene bag (33 × 38 × 0.02 mm) and sterilized at 121°C for 90 minutes. After cooling to 20°C, about 100 g inoculum was inoculated in a sawdust medium. The inoculated media were incubated in dark room 35 days at 22 ± 2°C and duration of mycelial growth, mycelial density were examined. The five kinds logs were cut 20 cm in length, 12~15 cm in diagram and put into polypropylene bags. Sterilization and inoculation were made by the same method as described previously.

Experimental condition. After the completion of spawn run (35 days), the upper 70% part of polypropylene bags were removed and the synthetic media were placed in cultivation house. Relative humidity was maintained 80 to 90% during initiation of primordium and 60 to 70% during fruiting body growing. The temperature was maintained at 26 to 32°C throughout the experiments. The fruiting body yields of *P. gilvus* mushroom on various sawdust media displayed wt. of dried individual fruiting body (g), length of pileus (mm), width of pileus (mm), thickness of pileus (mm), wt. of fresh fruiting body (g) and wt. of dried fruiting body (g).

Antitumor activity. Antitumor activity measured by the sulforhodamine B (SRB) assay (Kim *et al.*, 1996) using murine P388 cells. The 180 *μl* RPMI 1640 medium containing 1×10^4 murine P388 cells added to each well of 96-well format and incubated at 37°C, 5% CO_2, 24 hours. 20 *μl* extract of *P. gilvus* growing on various substrates added to each well of 96-well format and incubated at 37°C, 5% CO_2, 48 hours. Cultures fixed with trichloroacetic acid were stained for 30 minute with 0.4% sulforhodamine B in 1% acetic acid. Unbound dye was removed by five washes with 1% acetic acid and protein-bound dye was extracted with 10 *μ*M unbuffered Tris base. Absorbance was measured at 540 nm using a microplate reader.

Results and Discussion

Physico-chemical analysis. Physico-chemical analysis of medium resources, natural property investigation on each material: Chemical investigation showed us almost similar value of T-C percentage as like 46.6% of oak sawdust, 47.5% of mulberry sawdust, 46.3% of elm sawdust, 47.8% of acacia sawdust, and 43.2% of apple tree saw-

Table 1. Chemical compositions of substrates

Substrate	pH (1 : 5)	T-C[a]	T-N[b]	C/N	P_2O_5	K_2O	CaO	MgO
					(%)			
Oak sawdust	6.0	46.6	0.28	166	0.07	0.22	0.92	0.11
Mulberry sawdust	6.5	47.5	0.37	128	0.06	0.35	0.84	0.07
Elm sawdust	6.6	46.3	0.32	145	0.05	0.25	1.79	0.05
Apple sawdust	6.1	43.2	0.38	114	0.09	0.31	0.82	1.22
Acacia sawdust	6.3	47.8	0.43	111	0.05	0.16	0.98	0.06

[a]Total carbon, [b]Total nitrogen.

Table 2. Trace element compositions of substrates

Substrate	Fe	Mn	Cu	Zn	Pb	Cd	Cr	As
	ppm							
Oak sawdust	92.0	140.5	2.6	2.67	0.92	–	–	1.53
Mulberry sawdust	62.3	8.5	1.2	3.12	–	–	–	–
Elm sawdust	61.0	8.09	2.0	5.77	–	–	–	4.27
Apple sawdust	90.2	41.3	2.4	8.84	–	–	–	–
Acacia sawdust	112.8	23.2	2.1	5.06	2.59	–	–	0.14

Table 3. Comparison of mycelial growth, mycelial density and primordium formation of *P. gilvus* with various medium

Substrate	Duration of mycelial growth (days)	Mycelial density[a]	Days for pinhead formation
Oak tree	56	+++	12
Oak sawdust	28	+++	11
Mulberry tree	54	+++	13
Mulberry sawdust	27	+++	13
Elm tree	60	++	14
Elm sawdust	30	++	14
Apple tree	57	+++	13
Apple sawdust	29	+++	11
Acacia tree	63	++	15
Acacia sawdust	33	++	14

[a]Mycelial density: ++ = low, +++ = medium, ++++ = high.

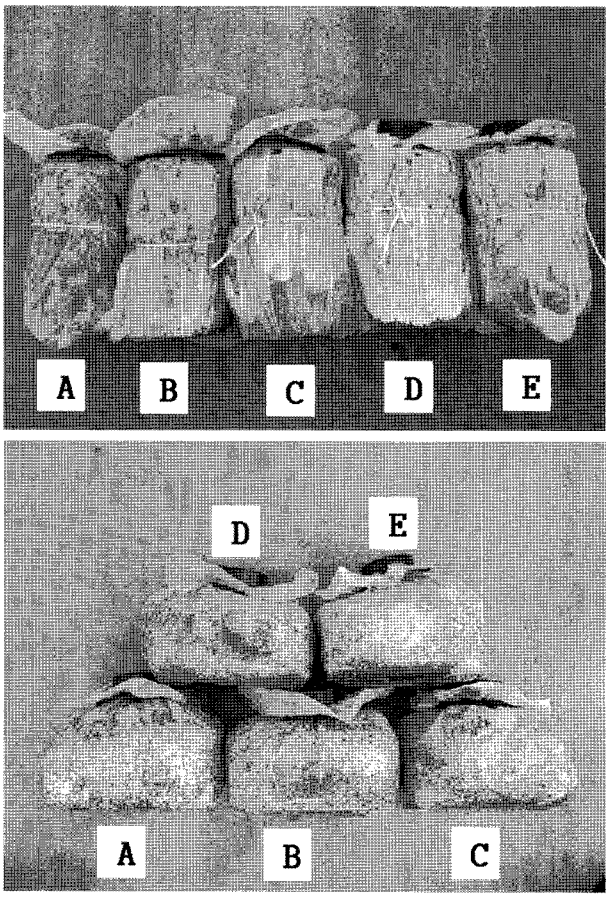

Fig. 1. Effect of timber and sawdust substrates on mycelial growth of *P. gilvus* KCTC 6653 at 25°C. A, Oak; B, Mulberry; C, Elm; D, Apple; F, Acasia.

dust. Their pH value was 6.0 of oak sawdust, 6.5 of mulberry sawdust, 6.6 of elm sawdust, 6.3 of acacia sawdust and 6.1 of apple tree sawdust. It is weak acidity for oak sawdust, apple tree sawdust, neutrality for mulberry sawdust and elm sawdust (Table 1).

A heavy metal examination showed that value of Fe was 112.8 ppm of acacia sawdust, 61.0 ppm of elm sawdust, and their Mn value was 140.5 ppm of oak sawdust, 8.5 ppm of mulberry sawdust, and Cu value was 2.6 ppm of oak sawdust, 2.0 ppm of elm sawdust, and Zn value was 5.77 ppm of elm sawdust, 2.67 ppm of oak sawdust, and Pb value was 0.92 ppm of oak sawdust, 2.59 ppm of acacia sawdust, none of mulberry sawdust, elm sawdust, apple tree sawdust. As value was 1.53 ppm of oak sawdust, 4.27 ppm of elm sawdust, 0.14 ppm of acacia sawdust. Cd and Cr were not detected in all substrates (Table 2).

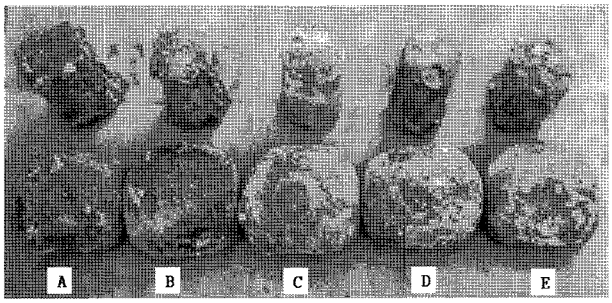

Fig. 2. Effect of substrate on primordium formation of *P. gilvus* KCTC 6653. A, Oak; B, Mulberry; C, Elm; D, Apple; F, Acasia.

Table 4. Effect of substrate on the fruitbody yields of *P. gilvus* KCTC 6653

Substrate	Wt. of dried individual fruitbody (g)	Length of Pileus (mm)	Width of Pileus (mm)	Thickness of Pileus (mm)	Wt. of fresh fruitbody (g)[a]	Wt. of dried fruitbody (g)[b]
Oak tree	11.1 ± 8.48[c)]	86.9 ± 27.61	48.9 ± 9.71	3.08 ± 0.28	179.0 ± 35.34	76.4 ±1 3.25
Oak sawdust	6.7 ± 3.25	81.9 ± 18.51	49.3 ± 8.93	2.68 ± 2.01	227.5 ± 58.55	84.2 ±1 7.12
Mulberry tree	4.5 ± 0.97	67.0 ± 8.84	41.1 ± 4.45	2.70 ± 0.34	106.8 ± 47.45	45.6 ± 18.84
Mulberry sawdust	8.7 ± 4.47	95.4 ± 21.90	54.1 ± 11.61	2.55 ± 0.51	93.4 ± 19.77	36.0 ± 6.94
Elm tree	3.3 ± 0.50	58.3 ± 6.02	30.3 ± 3.86	2.95 ± 0.26	21.3 ± 7.36	10.3 ± 2.62
Elm sawdust	4.6 ± 2.44	64.6 ± 14.99	42.1 ± 5.32	2.51 ± 0.41	76.1 ± 16.33	36.4 ± 4.66
Apple tree	3.7 ± 1.56	63.8 ± 13.36	35.4 ± 5.85	2.56 ± 0.25	106.6 ± 37.42	45.6 ± 16.07
Apple sawdust	8.7 ± 7.72	85.5 ± 20.18	48.5 ± 6.99	2.57 ± 0.73	170.2 ± 40.66	64.5 ± 8.04
Acacia tree	2.6 ± 1.98	57.2 ± 20.68	30.4 ± 6.84	2.40± 0.22	25.2 ± 10.35	8.4 ± 3.91
Acacia sawdust	4.5 ± 1.58	71.3 ± 11.84	43.7 ± 3.98	1.96 ± 0.25	108.9 ± 29.13	37.4 ± 9.13

[a]Weighed as fresh weight, [b]Weighed as dried weight, [c]Results are mean ± standard deviation of ten replicates.

Growth of mycelium in various sawdust substrate. To study for the possibility of artificial cultivation of *P. gilvus*, we investigated *P. gilvus* mycelium growing status with five kinds of sawdust or material lumber. It took about 2-times longer period of 54~63 days on cultivation with material lumber than 27~33 days on cultivation with sawdust until completing mycelial growth (Table 3, Fig. 1). Mycelial density on elm sawdust and acacia sawdust were lower than those of oak sawdust, apple sawdust. These result was similar that mycelial growth of *P. linteus* on oak sawdust, apple tree sawdust, peach tree sawdust were good, whereas acacia sawdust was poor (Chi *et al.* 1998). The days for primordium formation showed us almost similar as 11 days on oak sawdust and apple tree sawdust, 13 days on mulberry sawdust and apple tree (Fig. 2).

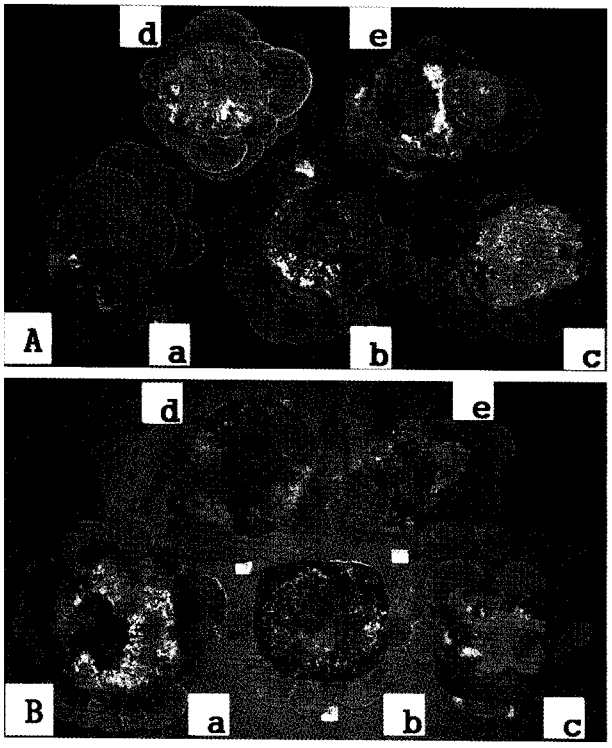

Fig. 3. Effect of substrate on fruitbodies of *P. gilvus* KCTC 6653. A, Cultivation on log; B, Cultivation on sawdust. a, Oak; b, Mulberry; c, Elm; d, Apple; e, Acasia.

Character of fruiting body in various sawdust substrate. Weight of dried fruiting body showed that 76 g on oak tree, 84 g on oak sawdust, 10 g on elm tree, 36 g on elm sawdust, 46 g on apple tree, 65 g on apple sawdust. Weight of fresh fruiting body showed that 179 g on oak tree, 227 g on oak sawdust, 21 g on elm tree, 76 g on elm sawdust, 106 g on apple tree, 170 g on apple sawdust. Among them, the yield of oak substrates was the highest whereas acacia sawdust was the poorest. Rew *et al.* (2000) reported that the wt. of fresh fruiting body was 180 ± 86 g on oak tree, 150 ± 15 g on apple tree and 29 ± 8 g on pine tree. This is in accord with our experimental results, and it is concluded that the yields of sawdust substrates were higher than log substrates. Thickness of pileus (mm) showed that 3.1 mm on oak tree, 2.7 mm on oak sawdust, 2.9 mm on elm tree, 2.5 mm on elm sawdust and 2.5 mm on apple tree and 2.5 mm on apple sawdust. These results showed that the thickness of pileus on

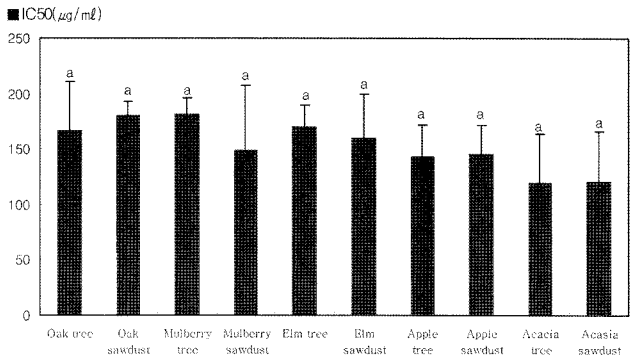

Fig. 4. Anti-cancer activity against P388 cell line of *P. gilvus* KCTC 6653 grown on the various tree and sawdust. Superscript (a) indicate significant difference ($P < 0.05$).

log substrates was thicker than sawdust substrates. The length of pileus (mm) showed that 87 mm on oak tree, 82 mm on oak sawdust, 67 mm mulberry tree, 95 mm on mulberry sawdust, 58 mm on elm tree and 65 mm on elm sawdust (Table 4, Fig. 3).

Antitumor activity. Extract of many *Phellinus* spp. have an anti-tumor activity but a little is known regarding the anti-tumor activity of *P. gilvus* (Bae *et al.*, 2004). By SRB assay, *P. gilvus* grown on sawdusts and logs of oak, mulberry, elm, apple, acacia showed similar in anti-tumor activity (Fig. 4).

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