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Utilization of *Robinia pseudoacacia* as Sawdust Medium for Cultivation of Edible and Medicinal Mushrooms

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ABSTRACTS

This study was undertaken to examine the feasibility of black locust (*Robinia pseudoacacia*) as substrates for several edible mushrooms. For the cultivation of several edible and/or medicinal fungi on black locust, optimum bulk densities, synthetic or semisynthetic additives, natural additives and pretreatment methods were investigated. Fruit body yields of the fungi on various sawdust media composed of different wood species were also analyzed for testing the capability of black locust as a substrate for mushroom production.

Mycelial growths decreased proportional when the bulk density increased. The most suitable carbon and nitrogen sources as additives to promote the mycelial growth were sucrose (2%, w/w) and ammonium phosphate (0.2%, w/w) respectively. When corn-powder and beer-waste as natural additives were added to sawdust of black locust showed the significant growth of mycelia. And the optimum mixing ratio was 10:2:1 (sawdust: corn-powder: beer-waste, w/w). Black locust after cold water treatment showed the outstanding mycelial growths. Any significant changes of pH, moisture content (%) and dry-weight losses (%) could not be found among culture substrates (sawdust of black locust, oak and poplar wood) examined before and after harvesting of fruit bodies. Yield of fruit bodies on black locust culture media were comparable with those culture media composed with oak and poplar wood. The present work indicated strongly the potentiality of black locust as raw materials for edible and medicinal mushrooms.

Key Words *: black locust, edible mushroom, bulk density, carbon and nitrogen sources, natural additives, cold water treatment.*

INTRODUCTION

Black locust (*Robinia pseudoacacia*) is known as an excellent source for the production of good dimension of lumber. Furthermore, it provided quick stabilization of disturbed sited, enhanced soil quality by furnishing nitrogen and nutrient litter and fosters the development of high quality fostered stands (Han, 1991). Thus black locust in Korea was planted extensively for erosion control and for land reclamation purpose during 50' and 60' . Afforestation area of black locust in Korea is estimated about 325,000 ha which is approximately 16% of the total afforestation area. However, most of plantations has not been managed properly. Hence, small diameter of black locust under 15cm dominates the total production

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of black locust in Korea. Unfortunately, black locust has been traditionally regarded as unfavorable wood species in wood industries due to its difficult properties in wood processing (Park, 1996).

Since logs for mushroom cultivation has diminished quickly in recent years, the shortage of hardwood has become a serious problem in field of mushroom industries. Oak wood species (*Quercus* spp.) traditionally have been the preferred substrate for the cultivation of Shiitake mushroom (Singer and Harris, 1987). As a result of the rapid expansion of cultivation of Shiitake, however, it is now very hard to get the log of oak wood for Shiitake cultivation. Hence, other tree species have been examined alternatively for the cultivation of edible mushroom to overcome this crisis. Although softwood sawdust has been tried, many edible mushroom can not utilize it as effectively as hardwood species since extractives in heartwood of softwoods inhibited the mycelial growth of edible mushroom (Park et al., 1994)

Although many hardwood species has been tried for cultivating edible mushroom, however, black locust has rarely been attempted for the cultivation of edible mushroom until now. The present work was hence, undertaken to examine the feasibility of black locust as medium for the cultivation of edible and medicinal mushroom.

METERIALS AND METHODS

MATERIALS

Organisms: *Lentinus edodes, Pleurotus ostreatus, Flammulina velutipes, Ganoderma lucidum, Grifola frondosa.*

Substrates: sawdust of *Robinia pseudoacacia, Quercus serrata, Populus* spp.

Natural additives: Rice-bran, Corn-powder, Beer-waste

Synthetic additives: Galactose, D-maltose, Mannose, Starch, and Saccharose as carbon source and Potassium Nitrate, Calcium Nitrate, Sodium Nitrate, Asparagine and Peptone as nitrogen sources.

METHODS

Preparation of Media

Basal sawdust medium was consisted of sawdust (10~20mesh) of black locust, fresh rice-bran and corn-powder in the ratio of 5:1:1(w/w). After watering (65% before autoclave), these mixtures were each filled into the autoclavable polypropylene bottle (85mm diameter, 1,000ml) or the tube (diameter 24 mm) and compressed, keeping the bulk density 0.23 (g/cc). Thereafter, tubes or bottles plugged with cotton or screwcap were sterilized at 121℃ for 30 min and then allowed to cool to room temperature. Inoculation plugs (discs of 4mm diameter) for each fungal strains cut out from the margins of young colonies of 15-day-cultures grown on a potato dextrose agar (PDA, Difco Laboratories) were inoculated aseptically at the center of the each surface. Four additional discs were also inoculated between center and margin in four directions for bottle culture.

Bulk Density

After mixing the ingredients of basal medium and water, tubes were filled with the mixtures in such different bulk densities of each tubes as 0.19, 0.21, 0.23, 0.25 (g/cc). Linear growths were measured from the 3 days after inoculation through the colonization.

Effects of Carbon and Nitrogen Sources

Several carbon (2%, w/w) and nitrogen sources (0.2%) were supplemented to the basal medium. The mixtures were filled into the testing tube. After inoculation, linear growths were measured. Growth ratio (%) was determined as $A/B \times 100$; where A: the length of mycelial extension on the supplemented medium on the 20th day of incubation, and B: the length of mycelial extension of the basal medium.

Effects of Natural Additives

Three kinds of natural additives were mixed with sawdust of black locust in such ratios as shown in the legend of Fig. 2 and followed the process of basal media preparation. On the 20th day of incubation, the linear growths of fungi in the test tube were checked.

Pretreatment

100g of sawdust of black locust was pretreated with 3 *l* of cold (15-20℃, 24h), hot water (95~103 ℃, 6h) and methanol (65~70℃, 3h) for alternation of physiochemical characteristics of black locust. Filtrates (10~20mesh) was mixed with natural additives like as basal media. After inoculation, the linear growths in the test tube were checked.

Yields of fruit bodies

The productivity of black locust as substrates for edible mushroom was examined in comparison with other hardwood species (oak and poplar). Sawdust screened (10-20mesh) were mixed with natural additives (sawdust: rice-bran: corn-powder, 5:1:1, w/w) and watered (65%). After filling (0.23g/cc) into the bottle, the bottles were sterilized and cooled to the room temperature. After inoculation and incubation at 23~25℃, the cultural processes were followed. After certain incubating period, bottles were exposed to the new environment to promote fruit body production, in which temperature was lower and humidity was extremely higher and lightening, if necessary.

Determination of pH, Water Content and Dried-matter Losses

Before and after harvesting the fruit bodies, five cultures taken out of bottles were crushed and the pH, water content and dried-matter losses were determined.

RESULT AND DISCUSSION

It is well known that mycelial growth rate on substrates have a basic and decisive meaning in preparing the media for cultivation of mushroom because the early colonization of the medium by fungal mycelia may discourage the occurrence of disease or competitor fungi, at least, under the initial stage (Lee, 1991, Przyblowicz and donoghue, 1994). Mycelial growth on sawdust media filled with various bulk densities was different. Fig. 1 shows that the mycelial growth decreased proportionally when the bulk density increased. Sawdust media of black locust with bulk density 0.19 showed the best growths of all testing organisms.

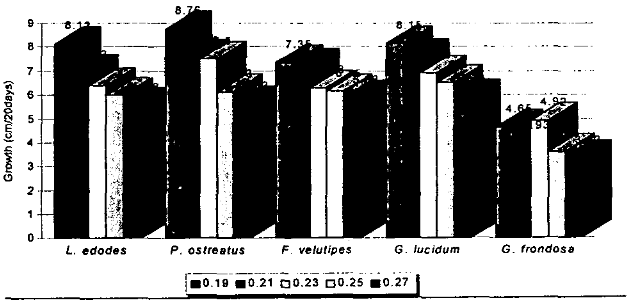

Fig 1. Mycelial growth of testing organisms on media with different bulk densities.

The present work suggests that the low level of compactness in bulk density would affect the porosity and the oxygen supply. The present work suggested also that air and/or oxygen played an important role of mycelial growth of edible mushrooms (lignin-degrading fungi in the present work) on black locust sawdust media. It is well known that lignin degradation is dependent on oxygen for different enzymes to be active (Eriksson et al. 1990) and that there was a direct correlation between the bulk density of media and mycelial growth of fungi (Kang et. al., 1989). It should be noted, however, that media with bulk density 0.23 is being widely employed as the optimum bulk density in the edible mushroom industry. Further studies are needed that the optimum bulk density for promoting the mycelial growth would be varied on sawdust substrates or not.

To obtain the most suitable culture conditions for promoting the growth of fungal mycelia, the influence of various carbon, nitrogen sources and natural additives on the mycelial growth was examined. Depending upon the fungal strains, demand for suitable carbon and nitrogen varied a little. Carbon sources promoting the linear growth were sucrose and mannose for *L. edodes*, saccharose and mannose for *P. ostreatus*, galactose for *F. velutipes*, sucrose and galactose for *G. lucidum* and starch for *G. frondosa*. Leatham(1986) found that addition of glucose, fructose or xylose stimulated ligninolytic activity when the fungus of *L. edodes* was grown on an oak wood sawdust medium.

In the nitrogen compounds, peptone and calcium nitrate were suitable for *L. edodes*, ammonium nitrate and asparagine for *P. ostreatus*, sodium nitrate and asparagine for *F. velutipes*, asparagine and peptone for *G. lucidum* and peptone for *G. frondosa* (Table 1). Even though there were some differences of carbon and nitrogen sources depending on the fungal species, sucrose (2%, w/w) and ammonium phosphate(0.2%, w/w) were overall the most suitable sources that could be widely employed for promoting the mycelial growth of fungi. It has been suggested that addition of small amount of nitrogen to certain fungal cultures may increase the efficiency of the fungi in utilizing lignin and lignin-related compounds(Eriksson et al. 1990).

Natural additives(rice bran, corn bran and beer waste) were added with various ratios, as a supplementation to sawdust of black locust for stimulating the growth of fungal mycelia and the production of fruit body. All the fungi examined showed the stimulation of growth when natural additives were supplied as shown in Fig. 2. In particular, when corn bran and beer waste were added, all the fungi showed the relatively significant growth of mycelia regardless of species of edible mushrooms. The optimal mixing ratio of natural additives to sawdust of black locust was 2:1:10 (Corn bran: beer waste: sawdust, w/w). It is interesting to note that rice bran did not show any significant effect on the mycelial growth as revealed in *G. lucidum* and *G. frondosa*.

Black locust is well known to have a higher amount of extractives in heartwood (Fengel and Wegener 1983);

Table 1. Effect of carbon sources on the mycelial growth of testing organisms

Additives \ Fungi		Growth Ratio (%)				
		L. edode	P. ostreatus	F. velutipes	G. lucidum	G. frondosa
Carbon sourcesa[a)]	Sucrose	130	131	120	122	111
	Glucose	125	108	124	102	115
	Fructose	110	106	119	109	102
	Mannose	137	140	110	101	96
	Galactose	124	109	150	120	85
	Saccharose	106	142	131	121	101
	D-maltose	105	110	128	100	102
	Starch	110	107	101	90	140
Nitrogen sources[b)]	Ammonium phosphate	106	133	113	105	102
	Ammonium sulfate	102	142	117	97	100
	Potassium nitrate	96	87	120	101	95
	Calcium nitrate	129	107	105	103	97
	Sodium nitrate	114	107	132	104	99
	Asparagine	92	145	130	110	80
	Peptone	130	99	103	115	108

Notes. Growth Ratio (%) = A/B $\times$ 100. A : Length of mycelial extension on the medium supplemented on the 20th day of incubation, B : Length of mycelial extension on the basal medium. a) 2% (w/w) b) 0.2% (w/w)

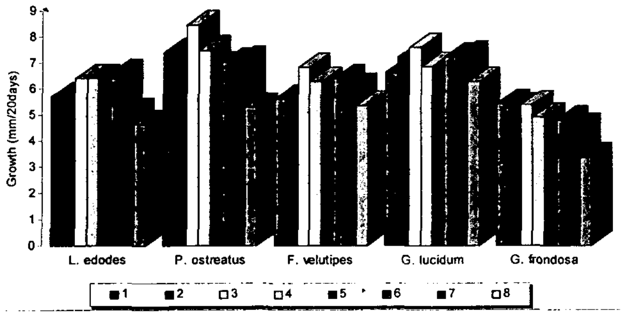

Fig 2. Mycelial growth of testing organisms on media supplemented with various natural additives with different ratios.
The mixing ratios of sawdust: rice-bran: corn-powder: beer-waste;
1, 10:2:2:1, 2, 10:2:0:1, 3, 10:0:2:1, 4, 10:2:2:0, 5, 10:2:0:0, 6, 10:0:2:0, 7, 10:0:0:1, 8, 10:0:0:0

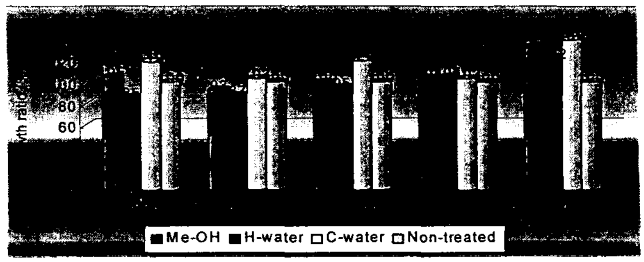

Fig 3. Effects of various pretreatment of sawdust on the linear growth of testing organisms.

Most of them are phenolic compounds. Since the content of extractives in black locust is especially higher than any other hardwoods, it has been though that these extractives may be detrimental to the fungal growth. Biodegradability of lignocellulosic materials can be enhanced by physical, chemical and biological pretreatment. In the present work, sawdust of black locust was treated with cold and hot water and organic solvent (methanol) to enhance the fungal growth in black locust. Quite interestingly, the promoted mycelial growth was shown in sawdust media pretreated with cold water even though there were some differences of growth among fungal species. In particular, *G. frondosa* showed the enhanced mycelial growth regardless of procedures of the pretreatment. In contrast, hot water extraction did have little effect on the mycelial growth.

Our result, however, did not agree with that of Park et al. (1994), reporting that hot-water extraction was the most effective for the fungal growth of *L. edodes*. This difference would be ascribed to the wood species extracted. Since they used the softwood species such as larch, pine, it is expected that the kinds of extractive between softwood and hardwood should be different. It has been suggested that low molecular weight of sugars, phenolics and cations are extracted with cold water (Fengel and Wegener, 1983). The present work indicated that the effect of pretreatment with water and organic solvents upon the growth of fungi was different with the fungal species. Furthermore the present work suggested also that the hot-water extractives of black locust did not play an important role in the fungal growth.

The variations of the pH, dry matter, moisture content of substrates were examined for monitoring the physicochemical characteristics of black locust during the production of fruit bodies. The pHs of the substrates before inoculation (as control) were 6.5 (*Robinia*) and 6.8 (*Quercus, Populus*), respectively. However, before and after harvesting the fruit bodies of *L. edodes*, the value of pH was 3.7 and 3.9 in black locust media, 3.8 and 3.6 in oak wood media and 3.9 and 3.9 in poplar wood media respectively (Table 2). Our results indicated that there were not any significant changes of pH before and after harvesting the fruit bodies. The present work indicated, however, that there was a drastic change of pH values during the incubating period of *L. edodes* because it dropped from 6.5 to 3.8. Our result indicated that the pHs of the substrates colonized by the testing organisms decreased continuously until the pre-harvest and thereafter it remained constant. It is well known that the fungi are able to adjust the pH values by forming acids or ammonia for the purpose of forming the optimum pH value during a certain stage of development (Han et al., 1981; Jablonsky, I., 1981) that affected the hydrogen ion concentration.

It should be mentioned, however, that there were somewhat differences of pH values among the testing organisms and substrates before and after harvesting as presented in Table 2. It has been widely known that organic acids and water in the media could be accumulated during the bioconversion process by mycelial mass and during the transference of mycelial mass into fruit bodies. And these changes varied with individual species, strain and even isolate and media (Kalberer, 1995; Jablonsky, 1981; Treschow, 1944). In addition, our results could be used as the index for deciding and predicting the timing of fruit-body initiation. Tokimoto and Komatsu (1978) reported that a pH between 3.5 and 4.5 was optimal for primordia formation and fruit body growth of *L. edodes.*

Table 2. Change of moisture content, pH, dry-weight losses before and after harvesting the fruit bodies

			pH	Dry-matter(%)	Moisture Content
Lentinus Lentin	*Robinia*	Pre-	3.7	75	63
		Post-	3.9	57	41
	Quercus	Pre-	3.8	70	63
		Post-	3.6	54	41
	Populus	Pre-	3.9	72	62
		Post-	3.9	60	42
Pleurotus	*Robinia*	Pre-	4.6	94	62
		Post-	4.7	82	41
	Quercus	Pre-	4.8	94	63
		Post-	4.5	81	42
	Populus	Pre-	4.7	92	62
		Post-	4.9	85	44
Flammulina	*Robinia*	Pre-	5.8	92	64
		Post-	5.9	68	56
	Quercus	Pre-	5.3	95	64
		Post-	5.3	69	57
	Populus	Pre-	5.8	92	64
		Post-	5.9	68	56
Ganoderma	*Robinia*	Pre-	4.4	94	61
		Post-	3.9	60	56
	Quercus	Pre-	4.5	94	63
		Post-	3.9	60	58
	Populus	Pre-	5.0	95	62
		Post-	4.3	73	58
Grifola	*Robinia*	Pre-	4.6	94	64
		Post-	4.6	75	57
	Quercus	Pre-	4.6	96	63
		Post-	4.8	60	57
	Populus	Pre-	4.7	95	62
		Post-	4.6	60	56

Pre- : Pre-harvest, Post- : Post-harvest
Data are means of five replicates.

Dry matter decreased continuously until the initiation of fruit body while moisture content did not show any changes. In contrast, the pH of media remained constant after harvesting the fruit body whereas dry matter and moisture contents decreased significantly as shown in Table 2. It has been reported that the greater biomass, increased levels of enzymes present in the substrates, increased solubility of substrates, and increased metabolic activities would resulted in the increased moisture content (Ohga, 1992).

Fruit bodies yields on three different culture media composed with different wood species showed little difference ($P<0.05$) as shown in Table 3. *F. velutipes* and *G. frondosa* showed the highest yield on black locust media. In contrast, *L. edodes* and *G. frondosa* showed the highest yields on oak media whereas *P. ostreatus* showed the highest on poplar wood media. The present work indicated that the yield of fruit body was different depending upon the organisms and culture media of wood species. However, differences

Table 3. Fruitbody yields of testing organisms on various media composed of three tree species.

		L. edodes	*P. ostreatus*	*F. velutipes*	*G. lucidum*	*G. frondosa*
Yields (g)[a]	*Robinia*	18.17±0.57[b]	4.84±0.64	12.29±0.73	5.77±0.50	5.12±0.63
	Quercus	19.03±1.16[c]	5.73±0.79	11.95±1.95	6.09±0.93	4.12±0.37[c]
	Populus	17.30±1.21	6.54±0.72[c]	10.76±0.88	6.67±1.01	4.48±0.52

a Means of 10 replicates
b Standard deviations of 10 replicates
c Significantly different Robinia sawdust medium in their capacity of fruitbodies at the 0.05 probability level.

were negligible; no marked differences of fruit body yield could be found in black locust media when compared to oak wood and poplar wood media.

Taken together no significant differences were found in moisture content, pH and dry weight losses in culture media composed of black locust when compared with other wood species that were traditionally used for the cultivation of mushroom. Furthermore the yield of fruit body cultured on sawdust of black locust media were comparable with those cultured on oak and poplar wood. Present work suggested strongly that the black locust can be used as an alternative substrate to culture the edible fungi when some additives were supplemented. Our results also suggested that small diameter of black locust woods can be converted efficiently into value-added products. Further studies are planned to establish an optimal condition for the production of edible mushrooms using black locust sawdust media.

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