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Sawdust Media Affecting the Mycelial Growth and the Fruiting Body Formation of *Sparassis crispa*

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Six strains of *Sparassis crispa* such as *S. crispa* DUM-01, DUM-02, DUM-03, DUM-04, DUM-05, and DUM-06 were tested for their mycelial growth on 6 different kinds of sawdust media and primordial formation on 10 different compositions of larch sawdust media. The highest mycelial growth was recorded on the larch sawdust. Of the 6 strains of *S. crispa*, *S. crispa* DUM-04 recorded the favorable formation of primordia. The primordial formation of *S. crispa* DUM-04 was more favorable on L-3 medium than 9 kinds of larch sawdust media. When *S. crispa* DUM-04 was cultured on the media of larch sawdust + pine sawdust, the formation of its fruiting bodies was more outstanding on the media of larch sawdust + pine sawdust than those of larch sawdust.

KEYWORDS: Fruiting bodies, Mycelial growth, Primordia, *Sparassis crispa*

Sparassis crispa, one of the edible and butt-rot fungi belongs to *Coriciaceae* of *Basidiomycetes* (Igarashi and Takeuchi, 1985; Kim, 1991; Mao and Jiang, 1993). *S. crispa* has been reported to cause a brown root-and butt-rot in living conifers, closely similar to damage caused by *Phaeolus schweinitzii* (Igarashi and Takeuchi, 1985; Woodward *et al.*, 1993). Also, *S. crispa* has been reported to cause an active wood destruction in blocks of Douglas fir such as sapwood or heartwood (Delatour, 1975; Kim, 1993; Ohga, 1998). However, *S. crispa* has been known to produce an antifungal metabolite such as parassol (methyl-2hydroxy-4 methoxy-6-methyl benzonate) against *Cladosporium cucumerinum* causing foliar diseases (Wedekind and Fleischer, 1923; Woodward *et al.*, 1993). Particularly, the fruiting bodies of *S. crispa* were reported to exhibit an outstanding effect for curing human diseases such as a gastric ulcer, esophageal cancer, hypertension and diabetes (Mao and Jiang, 1993). The β-glucan contained in the fruiting body of *S. crispa* was observed to have the capacity to activate human leukocytes and related immune system (Nameda *et al.*, 2003). Since 1923, various researches of *S. crispa* have been carried out intensively to control human diseases or foliar diseases (Wedekind and Fleischer, 1923; Igarashi and Takeuchi, 1985; Kim, 1991; Woodward *et al.*, 1993; Kim, 2000). To satisfy various many users demands for edible fungi of *S. crispa*, one of urgent problems must be focused on the mass production of fruiting bodies. Shim *et al.* (1998) studied the optimal factors for the mycelial growth of *S. crispa*. This study was carried out to select sawdust media suitable for stimulating mycelial growth and forming fruiting bodies of *S. crispa*.

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Materials and Methods

Fungi used. As shown in Table 1, six strains of *S. crispa* were inoculated on potato dextrose agar adjusted to pH 4 and incubated at 25°C under the dark condition (Table 1).

Selection of sawdust favorable for mycelial growth. To screen sawdust medium suitable for the mycelial growth of *S. crispa*, six different kinds of sawdust were prepared with oak (*Quercus mongolica*), mulberry (*Morus alba*), poplar (*Populus deltoides*), larch (*Larix kaemferi*), pine (*Pinus densiflora*) and Korean white pine (*Pinus koraiensis*). The sawdust was adjusted to the humidity of 70%, put into test tubes (dia. × length; 18 × 180 mm) and then autoclaved twice at 121°C for 30 minutes. A 5 mm

Table 1. *Sparassis crispa* isolates used in this study

Isolates	Origin	Location
S. crispa DUM-01	Dongguk University 1	Kwangreung, Korea
S. crispa DUM-02	Dongguk University 2	Kwangreung, Korea
S. crispa DUM-03	KFRI[1] 245	KFRI, Korea
S. crispa DUM-04	Dongguk University 4	Kwangreung, Korea
S. crispa DUM-05	CBS[2] 830.91	CBS, Netherlands
S. crispa DUM-06	KFRI[1] 69525	CBS, Netherlands

[1]KFRI; Korea Forestry Research Institute, Seoul, Korea.
[2]CBS; Centraalbureau voor Schimmelcultures, Netherland.

Table 2. Compositions of sawdust media

Culture media	Compositions (v/v, %)								
	L	RB	BL	R	W	C	P	WB	G
[a]L-1	100								
L-2	60	40							
L-3	60	20		20					
L-4	60	20			20				
L-5	60	20				20			
BL-1			100						
BL-2		40	60						
BL-3		20	60	20					
BL-4		20	60		20				
BL-5		20	60			20			
[b]Larch sawdust	60	20		20					
Larch sawdust + Pine sawdust	60						20	15	5

[a]Ten sawdust media for mycelial growth.
[b]Two sawdust media for forming fruiting body, L, Larch sawdust; BL, Boiled larch sawdust; RB, Rice bran; R, Rice; W, Wheat; C, Corn; P, Pine sawdust; WB, Wheat bran; G, Glucose.

diameter plug of an inoculum was transferred to each sawdust medium and incubated for 75 days at 25°C under the dark room condition maintained with RH of 70%. During 75 days, the measurement of mycelial growth has been performed at an interval of 5 days.

Selection of sawdust media effective to the formation of primordia. To screen sawdust media and isolates effective to the primordial formation of *S. crispa*, larch (*Larix kaemferi*) sawdust of several kinds of substrates most favorable to mycelial growth was chosen. Two kinds of larch sawdust, the unboiled and boiled larch sawdust were prepared. The unboiled sawdust was prepared with autoclaving twice at 121°C for 30 minutes, whereas the boiled sawdust was boiled at 100°C for 150 minutes, dehydrated at 80°C for 24 hours in the dry oven and autoclaved twice at 121°C for 30 minutes. In this experiment, the boiled larch sawdust was prepared to identify Fukushima's results (2001) that the boiled sawdust was more effective to mycelial growth of *S. crispa* than the unboiled sawdust. Except for L-1 and BL-1 media, all of 8 different larch sawdust media were manufactured by adding the different ratios of rice bran and cereals such as rice, wheat, and corn in the 250 *ml* flasks. Ten kinds of the larch sawdust media were adjusted to the moisture condition of 70% and autoclaved twice for 30 minutes at 121°C (Table 2). All kinds of sawdust media were adjusted to pH 4. A 5 mm diameter plug of mycelia was transferred to center of each sawdust medium and incubated for 10 weeks at 25°C under the dark room maintained with moisture condition of 70%.

Selection of sawdust media effective to the formation of fruiting body. *S. crispa* DUM-04 which recorded the highest primordial formation among 6 strains of *S. crispa* was chosen to test the formation of its fruiting bodies in this study. To form fruiting body of *S. crispa*, two kinds of sawdust media such as larch sawdust and larch sawdust + pine sawdust were manufactured. In case of the larch sawdust media, the media were chosen by results of some tests for forming primordia. The larch sawdust was manufactured in the ratio of 60% larch sawdust + 20% rice bran + 20% Rice. The larch sawdust + pine sawdust was prepared in proportions of 60% larch sawdust + 20% pine sawdust + 15% wheat bran + 5% glucose in the plastic bottles (850 *ml*). All kinds of sawdust media were adjusted at pH 4 (Table 2). A 5 mm diameter plug of mycelia was transferred to the center of each sawdust medium and incubated at 3 different temperature conditions for a total of 12 weeks such as 25°C for 6 weeks, 18°C for 1 week and finally 25°C for 5 weeks under the dark room condition maintained with RH of 70%.

Results and Discussion

Selection of Sawdust for mycelial growth. Of 6 kinds of sawdust, the mycelial growth of *S. crispa* except *S. crispa* DUM-04 was most outstanding on the larch sawdust media (Fig. 1). Since larch sawdust was more suitable to mycelial growth of *S. crispa* than any other sawdust, larch sawdust was selected to test the primordial formation.

Selection of sawdust media for formation of primordia. Since larch sawdust was identified as an effective substrate for stimulating mycelial growth of *S. crispa*, larch sawdust was selected to perform its primordial formation. Mixed with larch sawdust, the ratio of rice bran was based on Kang's method (1997), which adjusted the ratio of rice bran to 10~20%. Also, Jhune *et al.* (2000)

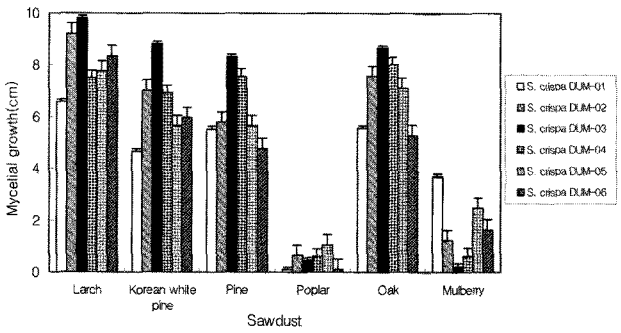

Fig. 1. Mycelial growth of *Sparassis crispa* in the different sawdust media at 25°C for 75 days.

recorded that *Pleurotus* spp. showed the highest mycelial density on the media containing rice bran of 20~25%. The primordia of *S. crispa* were formed favorably on L-3 media. Of 6 strains, *S. crispa* DUM-02, *S. crispa* DUM-04 and *S. crispa* DUM-05 were observed to form primordia. Of 3 strains, *S. crispa* DUM-04 was most favorable to the formation of primordia (Table 3). Although all the culture conditions were the same as those of all kinds of the media used in this investigation, the primordia were formed on L-3, L-4, L-5, BL-3 and BL-4. The mycelial growth of *S. crispa* was favorable on the boiled sawdust media, whereas its primordia were developed more on the unboiled sawdust media than the boiled sawdust. Although the mycelial growth of *S. crispa* was more favorable on boiled larch media than unboiled media as Fukushima's result (2001), it seems to be difficult to form primordia of *S. crispa* on the boiled sawdust media.

Selection of sawdust media for formation of fruiting body. It was observed that the fruiting body of *S. crispa* DUM-04 was formed on both larch sawdust and larch + pine sawdust (Table 4 and Fig. 2). As a result of comparing fruiting bodies, the fruiting body of *S. crispa* DUM-04 has been formed favorably on larch sawdust + pine sawdust during incubation period of 12 weeks (Fig. 2). The height and diameter of fruiting bodies recorded 59 and 55 mm on larch sawdust after 12 weeks of incubation, whereas 72 and 100 mm on larch sawdust + pine sawdust. The dry weight of *S. crispa* recorded 7 g and 3 g on larch sawdust + pine sawdust and larch sawdust, respectively. There was a significant difference ($p < 0.05$) in the formation of fruiting bodies between the larch sawdust and the larch + pine sawdust (Table 4). These results seem to imply that the larch + pine sawdust are more effective

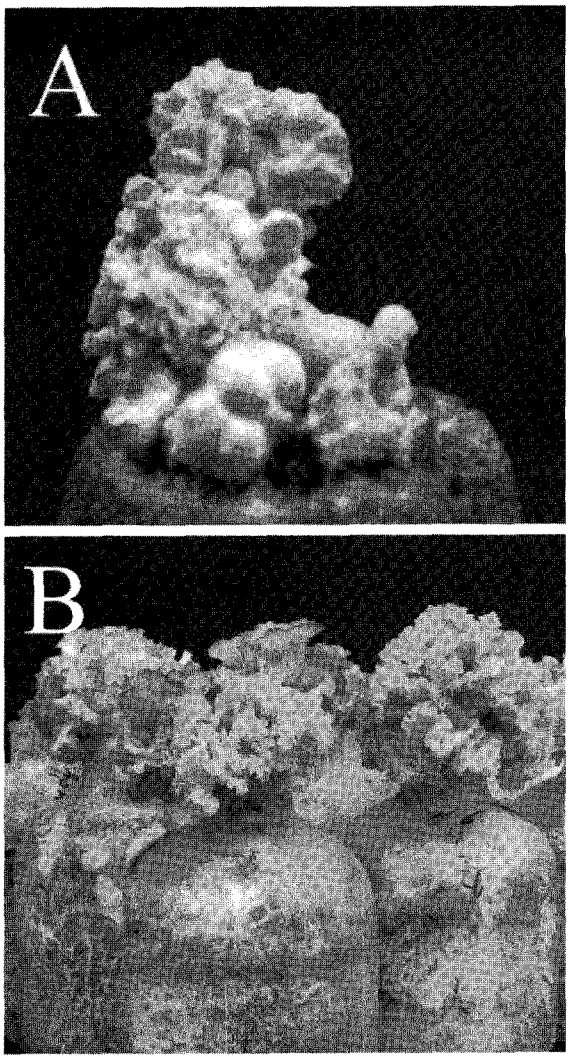

Fig. 2. Morphological characteristics of fruiting body of *S. crispa* DUM-04 in the larch sawdust (A) and the larch sawdust + pine sawdust (B).

Table 3. Primordia of *Sparassis crispa* formed in the different sawdust media

Strain	Culture media									
	L-1	L-2	L-3	L-4	L-5	BL-1	BL-2	BL-3	BL-4	BL-5
S. crispa DUM-01	–	–	–	–	–	–	–	–	–	–
S. crispa DUM-02	–	–	+	+	–	–	–	–	–	–
S. crispa DUM-03	–	–	–	–	–	–	–	–	–	–
S. crispa DUM-04	–	–	+++	+	+	–	–	+	+	–
S. crispa DUM-05	–	–	–	+	+	–	–	–	–	–
S. crispa DUM-06	–	–	–	–	–	–	–	–	–	–

–, No formation of primordium; +, primordial diameter less than 1 cm; ++, primordial diameter between 1 cm and 2 cm; +++, primordial diameter more than 2 cm.

Table 4. Fruiting body of *Sparassis crispa* formed in the sawdust media after 12 weeks of incubation at 25°C

Types of media[a)]	Fruiting body		
	Height (mm)	Diameter (mm)	Dry weight (g)
Larch sawdust	59	55	3.00
Larch sawdust + pine sawdust	72	100	7.00
Level of significance	0.4425*	0.0044**	0.0003**

[a)]The means of two sawdust media, within columns, are significantly different at ($p < 0.05$*) and ($p < 0.01$**), respectively.

to foming fruiting bodies of *S. crispa* than larch sawdust. Jo *et al.* (2002) reported that culture media of 90% oak + 10% rice bran were suitable for the formation of fruiting bodies of *Phellinus gilvus* and the total fresh weight of its fruiting bodies recorded 577 g equivalent to dried weight of 97 g. Jhune *et al.* (2000) reported that the fruiting body of *Pleurotus* spp. showed the highest yield in case of adding rice bran of 15~20% into a culture medium. In the experiment, the yield of fruiting bodies was recorded higher on larch sawdust + pine sawdust than larch sawdust. In the morphological characteristics of *S. crispa* (Lee, 1988), its fruiting body was in the range of 10~30 cm in diameter, and composed of tightly packed and flattened branches arising from a central stem. The branches with crisp ends were whitish to yellowish or tan in color and discolored gradually in advanced age. In our study, the fruiting body of *S. crispa* showed the range of 5.5 to 10 cm in diameter. Also, spore size of *S. crispa* was obtained in the range of 6.5~7 × 4.3~4.8 μm. Although the diameter of fruiting body was smaller than that of description on Lee's result (1988), other characteristics such as outer shape and spore size of *S. crispa* were very similar to those of description on Lee (1988).

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