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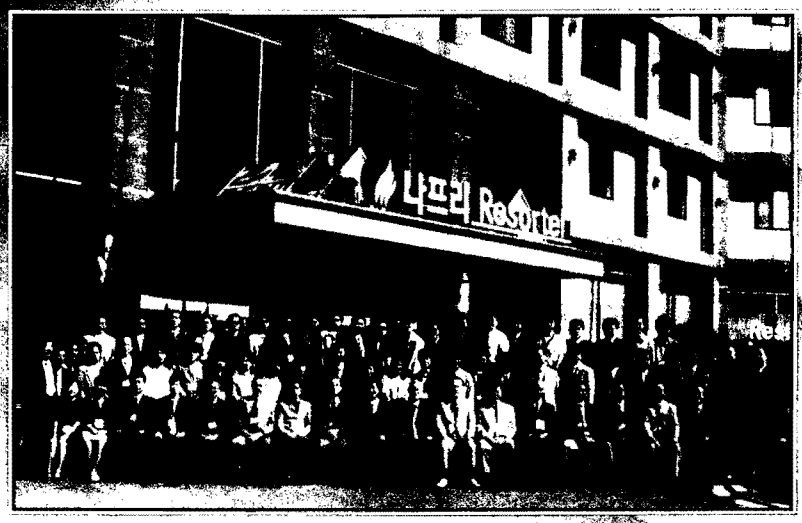

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인도네시아, 보고르, 보고르 농과대학교 생약센터

ABSTRACT : The objectives of this experiment were to study the growth and development of fruiting body of the two *Ganoderma lucidum* isolates on log of the soft wood *Paraserianthes falcataria* and the hard wood *Shorea* sp., and determination of organic germanium and crude ganoderic acid content of the fruiting body produced. The two *Ganoderma lucidum* isolates used were one Indonesian native (Indonesia isolate) and another isolate was purchased from Fungi Perfecti, USA (commercial isolate).

The development and quality of the primordium and fruiting body of the mushroom, in general, were influenced by the isolates used. The types of wood, however, had no effect on the quality of the primordium and fruiting body produced. The Indonesian isolate produced better fruiting body compared to that of the commercial isolate. The development of fruiting body from primordium, however, was low for the two isolates tested. In general, only about one third of the primordium developed further into mature fruiting bodies, except for the commercial isolate grown on the soft wood medium in which more than 60% of the primordium developed into mature fruiting body. Apart from producing normal fruiting body, the commercial isolate also produced an abnormal one, which had a white mature pileus, whereas the normal one was brownish red.

The organic germanium concentration of the fruiting body produced on the hard wood, in general, was higher than that of grown on the soft wood. The fruiting body from commercial isolate had higher organic germanium concentration compared to that of Indonesian isolate in both wood types. The two isolates used, however, had almost the same value of the crude ganoderic acid concentration in both types of wood tested. The Indonesian isolate had higher total yield of both organic germanium and crude ganoderic acid of the fruiting body produced compared to that of the commercial isolate.

KEYWORDS : *Ganoderma lucidum*, *Paraserianthes falcataria*, *Shorea* sp., mushroom primordium, fruiting body, organic germanium, and crude ganoderic acid.

INTRODUCTION

Ganoderma lucidum is one of the well-known medicinal mushrooms and it has been used for curing a variety of human diseases, particularly in China and Japan including hepatitis, nephritis, arthritis, bronchitis, cancer, hypertension, diabetes and gastric ulcers (Boh *et al.*, 2004; Wasser and Weis, 1999; Chang and Buswell, 1999). It has also been well documented that *Ganoderma lucidum* has diverse groups of bioactive compounds that play important role in medicinal and therapeutic properties; among of them are germanium and ganoderic acid (Boh *et al.*, 2000; 2004; Liu, 1999; Kim and Kim, 1999).

Ganoderic acid is one of triterpenoids that presence in *Ganoderma lucidum* and this compound has been received numerous research attention for its pharmacological effects and therapeutic value (El-Mekkawy *et al.*, 1998; Min *et al.*, 2000; Wagner *et al.*, 2003). Ganoderic acid is one of the useful metabolites which possesses

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anti tumor and anti HIV-1 activities.

Ganoderma sp. was reported to have high amount of germanium (Asai, 1980). Organic germanium plays important role in the stimulation of the immune system, antioxidant, anti-ischemic, anti-amyloidosis, and analgesic. The specific action of the organic germanium is on a biochemical level. The organic germanium renders oxygen availability of the cell that acts as a neutral semi-conductor in the electron transport processes inside the cells. This allows the formation of ATP and production of water (Goodman, 1988; Benjamin *et al.*, 1991).

Ganoderma sp. can be cultivated and can easily produce fruiting body in an artificial medium containing wood. First attempt to cultivate *Ganoderma* sp. using an artificial medium was conducted in 1937 and the cultivation of the mushroom can be carried out using wood containing medium, either as a sawdust or a log of wood (Mizuno, 1999; Chen, 1999). Even though the mushroom can be easily cultivated, but the functional substances of the fruiting body produced are influenced, among of them, by the type of medium (Tong *et al.*, 1991). It has been reported that the mushroom grown in the hard wood had higher content of germanium than that of grown in the soft wood (Chang, 1986).

Indonesia is one of the wood producer countries in the word. *Shorea* sp. and *Paraserianthes falcataria* are the important soft and hard woods, and grown abundantly in this country (Martawijaya, 1986; Nurhayati, 1988). Similarly, *Ganoderma* spp. including *Ganoderma lucidum* is one of the well-known mushrooms in Indonesia either as a plant pathogenic fungi, particularly in oil palm tree, or as a medicinal mushroom (Abadi, 1989; Purba, 1992), and they are often found in the two types of wood (Sukarno *et al.*, unpublished data). The attempts to cultivate Gonoderma lucidum for commercial used had been done in Indonesia, but so far are mainly focused on the fruit body biomass production. The quality of Gonoderma lucidum, however, is not only determined by the biomass but also by the bioactive compounds presence in the product (Boh *et al.*, 2004). Therefore, the experiment to study the effects of cultivation method of the *Ganoderma lucidum* on the production of its bioactive compound using local material for mushroom growth medium is required, hence this experiment. The aims of this experiment were to study the growth and development of *Ganoderma lucidum* on the hard wood *Shorea* sp. and the soft wood *Paraserianthes falcataria* and determination of organic germanium and crude ganoderic acid content of the fruiting body produced.

METHODS AND MATERIALS

Mushroom Species, Log Preparation, Cultivation Methods and Experimental Design

The two mushroom isolates examined in this study were *Ganoderma lucidum* isolated from Indonesia and an isolate collection of Fungi Perfecti, LLC, USA which is available commercially. This isolate will be referred as a commercial isolate in this paper. The cultures of the two mushrooms were obtained by isolating the mycelium from fresh fruiting body, which were grown on sawdust of *Paraserianthes falcataria*. The mycelium was cultured on Potato Dextrose Agar at 28 C for 5 days then transferred on sterile *Shorgum vulgare* seeds in 500 ml bottles and incubated at 28 C in the dark for 12 days prior to inoculating on the wood log.

The two types of log used in this experiment were the soft wood of *Paraserianthes falcataria* and the hard wood of *Shorea* sp. logs. Each log was having 15 cm in diameter. The logs were cut into 50 cm long and each side of the cut log was drilled to provide 28 holes. The woods were steamed for 10 hours in the 250 liters drum, then cooled and drained to remove the excess water by arranging the log standing for several days. Ten grams of each mushroom inoculum that previously grown on *Shorgum vulgare* were inoculated separately in each hole of each cut log, the holes then were covered by sterile cotton and followed by wax to prevent the inoculum from animal attacked and drying out. Each treatment was replicated three times. The logs were covered by a black polyethylene plastic to maintain the temperature and humidity of the logs, and incubated in the mushroom house. After the early stage of primordium developed, the plastics were removed and the logs were watered everyday using tap water. The observations on development of mushroom primordium and fruiting body were carried out daily by counting the number of primordium developed and number of fruiting body produced from primordium. After mature, fruiting bodies were harvested and the fresh weight and diameter were recorded. Dry weight was obtained after the fruit body

...s dried in the oven at 60℃ for 72 hours. The ...servations were carried out for 6 months after log ...culation. Completely randomized design with 4 ...atments and 3 replicates followed, in cases of ...nificance, by Duncan test ($P< 0.05$) were used to ...lyze the data in this experiment.

Extraction and Determination Procedures of Organic Germanium and Crude Ganoderic Acid

Organic germanium was determined using AAS ...omic Absorption Spectroscopy). Before the samples ...e subjected to AAS, the following treatments were ...ried out. The fruiting body was oven dried at 60 C ... 72 hours and grounded. Five grams of the grounded ...ing body were heated at 600 C for 3 hours, cooled ... 10 ml of hot 5N HCN was added into the sample ...wed by addition of double distilled water up to the ... volume of 25 ml. The residue of the sample was ...ed and the aqueous part of the sample was subjected ... AAS for determining of organic germanium ...centration (Anonymous, 1979).

Crude ganoderic acid was extracted by ethanol and ...roform. Ten grams of grounded fruiting body were ...racted in ethanol. The ethanol fraction was ...porated and extracted further by chloroform and ...er 1:1 (v/v). The chloroform fraction was ...rated then oven dried until reaching the constant ...ht (Chen and Chen, 2003).

RESULTS AND DISCUSSION

...e development of fruiting body of the mushroom ...itiated by the growth of mycelium, which will be ...ed by the production of mushroom primordium ...opoulos *et al.*, 1996). The time required to ...ce mushroom primordium after log inoculation ...vary (Table1) for both isolates tested in this experiment. In general, the production of primordium of commercial isolate was faster than that of Indonesia isolate in both types of wood. Commercial isolate produce primordium faster in the *Shorea* sp. compared to that of in the *Paraserianthes falcataria*. Commercial isolate required only a month to produce primordium in the *Shorea* sp., whereas in the *Paraserianthes falcataria* need more than 3 months after log inoculation. The two types of wood tested had no effect on primordium development of the Indonesian isolate. This isolate took more than 1 months after inoculation to produce mushroom primordium in both wood types tested.

The development of fruiting body from primordium was not different for the two isolates in the two types of growth medium, except for the Indonesian isolate in the hard wood of *Shorea* sp. in which required 3 times longer compared to the other treatments (Table 1). These findings were slower than our previous results using the sawdust of the two types of wood for the two mushroom isolates (Sukarno *et al.*, 2003).

The average number of primordium and fruiting body produced up to 6 months after inoculation were presented in Table 2. The two isolates produced almost the same number of primordium in the two medium types, however, further development of primordium to fruiting body was different in the two isolates. In general, more fruiting bodies developed from primordium in the commercial isolate. The results obtained in this experiment were much lower than that of in our previous investigation using the sawdust medium in which that almost all of the mushroom primordia were developed into fruiting body (Sukarno *et al.* 2003).

The quality of fruiting body, which was expressed as fresh weight, dry weight and diameter, was different significantly for both isolates tested. The Indonesian isolate had a better quality compared to the

...ble 1. The average of minimal time required for primordium development and production of fruiting body from primordium of the two *Ganoderma lucidum* tested.

Log	Primordium developed (Day after inoculation)		Fruiting body developed (Day after primordium developed)	
	G.lucidum (Commercial)	*G. lucidum* (Indonesia)	*G.lucidum* (Commercial)	*G. lucidum* (Indonesia)
...rea sp.	34.7b	122.7a	15.3a	50.8b
...raserianthes falcataria	96.7a	125.0a	17.2a	15.7a

... followed by the similar letter are not significantly different at $P< 0.05$.

Table 2. The average number of fungal primordium and fruiting body produced by *Ganoderma lucidum* per log in the duration of 6 months after inoculation*.

Log	Isolate	Number of Primordium	Number of Fruiting Body	Percentage of Primordium Developed to Fruiting Body (%)
Shorea sp.	*G. lucidum* (Commercial)	10.7a	3.7a	34.6a
	G. lucidum (Indonesia)	6.0a	1.7b	28.3b
Paraserianthes falcataria	*G. lucidum* (Commercial)	5.7a	3.7a	64.9c
	G. lucidum (Indonesia)	5.0a	1.7b	34.0a

Data followed by the similar letter are not significantly different at $P< 0.05$.
*Primordium and fruiting body produced after 6 months were not included.

commercial isolate in both wood log types (Table 3). Even though the indonesian isolate had low number of fruiting body, but it had a better quality in terms of dry weight and diameter. Comparing the data from this experiment using the wood log and our previous findings using the sawdust, it seemed that the Indonesian isolate grew better on the wood log whereas the commercial isolate was on the sawdust. In addition, commercial isolate produce abnormal fruiting bodies. They had a white mature pileus whereas the normal ones were brownish red. The abnormal mature pileus produced were about 6% and 11% of the total fruiting body production at the *Shorea* sp. and the *Paraserianthes falcataria*, respectively.

The concentration of organic germanium in the fruiting body was affected by the types of wood used (Table 4). There was a tendency that the organic germanium was higher in the isolates grown on the hard wood of *Shorea* sp. than that of on the soft wood of *Paraserianthes falcataria*. The differences were clearly observed in the Indonesian isolate. Indonesian isolate grown on the *Shorea* sp. had organic germanium concentration 3 times higher than that of grown on the *Paraserianthes falcataria*. The total yield of the organic germanium of fruiting body was, however, more determined by the biomass production rather than by isolate or type of wood. The values of the organic germanium obtained in this experiment were in the range of the published results reported by other workers. The reported values of germanium in the *Ganoderma* spp. were vary and range from 10 ppb to 1000 ppm (Tong *et al.*, 1991; and Asai, 1980).

Table 3. Quality of fruiting body produced by the two isolates of *Ganoderma lucidum* tested.

Log	Average Fresh Weight of Individual Fruting Body (g)		Average Dry Weight of Individual Fruting Body (g)		Average Diameter of Individual Fruting Body (cm)	
	G.lucidum (Commercial)	*G.lucidum* (Indonesia)	*G.lucidum* (Commercial)	*G.lucidum* (Indonesia)	*G.lucidum* (Commercial)	*G.lucidum* (Indonesia)
Shorea sp.	6.4a	26.3b	1.6a	10.0b	3.9a	7.0b
Paraserianthes falcataria	7.9a	30.1b	1.9a	9.6b	4.6a	8.3b

Data followed by the similar letter are not significantly different at $P< 0.05$.

Table 4. Concentration and total content of the organic germanium in the fruiting body per log of the two isolates tested.

Log	Concentration (ppm)		Total Content (mg)	
	G.lucidum (Commercial)	*G. lucidum* (Indonesia)	*G.lucidum* (Commercial)	*G. lucidum* (Indonesia)
Shorea sp.	77.4	6.68	0.01	0.07
Paraserianthes falcataria	5.37	2.25	0.01	0.02

The biomass of fruiting bodies was pulled due to not sufficient material for replication.

Table 5. Concentration and total content of the crude ganoderic acid in the fruiting body per log of the two isolates tested.

Log	Concentration (%)		Total Content (g)	
	G.lucidum (Commercial)	*G. lucidum* (Indonesia)	*G.lucidum* (Commercial)	*G. lucidum* (Indonesia)
Shorea sp.	2.05	2.61	0.033	0.260
Paraserianthes falcataria	2.03	3.11	0.041	0.309

: biomass of fruiting bodies was pulled due to not sufficient material for replication.

Table 5 shows the crude ganoderic acid concentration total content per log. The concentration of crude deric acid was not affected by either the isolate or pe of wood tested. Total yield of crude ganoderic n the other hand, depended on the isolate rather n the type of wood. The isolate that produced biomass, it will also have a higher total yield of rude ganoderic acid. The data obtained in this ment was higher than that of reported results on g body of *Ganoderma tsugae* (Chen and Chen, and on mycelium of *Ganoderma lucidum* (Tang ong, 2003) and fruiting body (Boh *et al.*, 2000). onclusion, the types of wood log as the growth e influenced the biomass and organic germanium tion of the two *Ganoderma lucidum* isolates . The two isolates tested produced moderate of organic germanium compared to the published . The cultivation method of the mushroom in periment need further improvement to produce etter quantity of the fruiting body. The crude ic acid content of the two isolates tested in this ent was high compared to the published reports need further analyzing since the compound l was the crude ganoderic acid.

적 요

의 목적은 참입수(*Paraserianthes falcataria*) 와 *Shorea*sp) 톱목에서 두 개의 *Ganoderma* 균주의 자실체 발생과 성장을 연구하고 자실체의 germanium 과 crude ganoderic acid 함량을 측 하이다. 두 개의 *Ganaderma lucidum*중 하나는 아 본지에서 분리한 것이고 또 다른 하나는 Fungi USA에서 구입한 것을 사용하였다. 일반적으로 서 생성된 자실체의 농도가 참입수에서 자란 것 높다. 두 종류의 톱목 모두에서 상업용 균주에서 자실체의 organic germanium 농도가 인도네시아 서 생산된 자실체의 organic germanium 농도보 . 이에 반하여 버섯 자실체의 crude ganoderic acid 농도는 두 개의 균주로부터 생산된 자실체에서 거의 같은 농도를 보였다. 인도네시아 균주는 상업용 분리균주와 비교했을 때 생산된 자실체의 총 organic germanium 과 총 crude ganoderic acid의 생산성이 높았다.

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