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Fungal Contaminant Threaten Oyster Mushroom (*Pleurotus ostreatus* (Jacq. ex Fr.) Kummer) Cultivation in Bali

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The main causal factor of the failure of the oyster mushroom (*Pleurotus ostreatus* (Jacq. Ex Fr.) Kummer) cultivation is baglog–borne fungal contamination which inhibit growth and production of oyster mushroom. Therefore this study was conducted to determine fungal contaminant in the baglog media and its inhibiting ability against oyster mushroom. The research was carried out by observation methods of sample randomly for 10–20% of the total contaminated baglog with three time replication. The results showed that air–borne fungus were potentially cause failure of oyster mushroom cultivation with the highest prevalence was *Fusarium* spp. (25.6%), while the highest inhibition ability was *Mucor* spp. (94.7±8.5%). The most dominant of baglog–borne fungal with its prevalence was *Trichoderma* spp. (35.71%). This fungus was very dangerous for the continuity of oyster mushroom cultivation in Bali.

Key words: oyster mushroom, inhibiting ability, fungal contaminants

INTRODUCTION

Development of oyster mushroom cultivation particularly in Bali is threated by a number of fungal contaminants. The fungal may origin from the air and sawdust media. Green mold caused by *Trichoderma* spp. is a major disease found in oyster mushroom cultivation (Kredic *et al*., 2010). Fungus isolated and characterized from compost were included *Aspergillus, Trichoderma, Mucor, Penicillium, Alternaria, Cladosporium, Monilia, Helminthosporium, Coccidioides,* and *Scedosporium* (Ashraf *et al*., 2007).

In Chiapas, Mexico, the most common contaminants during spawn phase were: *Streptomyces* sp., *Penicillium* sp. *Aspergillus ochraceus*, *A. flavus, Cunninghamella* sp., and *Trichoderma viride*. During the incubation phase were found *Monilia* sp. and *T. viride*. During fructification phase the most abundant contaminants were: *Poronia* sp. and *Coprinus* sp. (Lopez–Arevalo *et al*., 1996). In Indonesia, fungal contaminants were found to inhibit the growth of oyster mushroom was *Neurospora* spp., *Trichoderma* spp., *Mucor* spp., and *Penicillium* spp. (Anggrianto, 2012).

The quality of the growing media (compost), the number of spores in the air, and the phase of growth of oyster mushrooms determine the severity of contamination on baglog (Anastasi *et al*., 2005). The density of spores in the air, accompanied by a lot of opportunities for contamination causing the failure of oyster mushroom cultivation. Fungal contaminants that have been known to cause fungal growth failure of oyster mushroom were: *Neorospora* spp., *Trichoderma* spp., *Mucor* spp., and *Penicillium* spp. (Anggrianto, 2012). Fungal contaminants on the media type and inhibiting ability to the growth of oyster mushroom *in vitro* was unknown until now, therefore it is necessary for in–depth studies to answer the problems mentioned above.

MATERIALS AND METHODS

Baglog media preparation

The composition of the media used was wooddust: bran:corn:limestone (CaCO3):NPK at a ratio of 100:10:5: 2.5:1. Steps preparing baglog was: (1) all the ingredients were mixed while adding water. The amount of water was appropriated to medium compaction i.e. when clenched, it was not broken and its water was not squeezed until the baglog moved. (2) amount of 0.5 kg of medium fed into a heat resistant plastic size of 1 kg, and then pressed and closed it tightly with rubber slipping cotton on top. (3) Sterilized for 5 hours on 121°C, then stored in clean room, (4) a cold baglog was inoculated with mushroom seedling (F3) from prepared PDA in petri dish. The study was conducted in Breeding and Development Company Oyster Mushrooms located in Denpasar Bali from April, 2014 until November, 2014.

Oyster mushrooms seedlings preparation

A pure oyster mushrooms mycelia isolated from lamelle fruiting bodys in sterile PDA in Petri dish was considered as F1 and then subcultured to be F2 seedling in the same medium. F2 seedling inoculated into corn medium in a sterile bottle and it was considered as F3 which transferred to baglog medium cultivation.

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Inhibiting ability test of contaminants fungi against oyster mushrooms

Each contaminants fungus were tested for its inhibiting ability against the growth of oyster mushroom using dual culture technique. Inhibition percentage was calculated according to the following formula (Dolar, 2001; Jayalal and Adikaram, 2007; Mojica–Marin *et al*., 2008): Inhibiting ability = (A – B)/A x 100%; while A was diameter of oyster mushroom colony in the control (mm), B was diameter of oyster mushroom treated colony (mm).

Isolation and identification of air–borne fungi

Study of air–borne fungus was done by placing openly five Petri dishes containing PDA (made of 200 g potatoes, 15 g sugar, 20 g agar powder, 1000 ml distilled water) with 0.25% (w/v) livoploxasin (antibacterial substance) at the site of area research from 7:00 am to 13:00 pm. The fifth petri dish was closed after the exposed period and incubated for 2 days and the number of appeared colony fungus in Petri dishes were counted. Each colony was purified by subculturing to a new PDA and was incubated in the dark room at 27±2°C. Isolates were identified macroscopically according to colony color and growth rates, while the microscopically identification was done according to the hypha septation, spores/conidial shape and sporangiophore. The characters were checked to reference books of Samson *et al*. (1981), Pitt and Hocking (1997), Barnett and Hunter (1998), and Indrawati *et al*., (1999). Each different fungus species was tested for its inhibiting ability against oyster mushrooms with the procedure mentioned above.

Isolation and identification of baglog–borne fungi

Twenty percents of baglog were sampled from 50 pieces contaminated baglog. The baglogs sample were characterized by darker color at the top of baglog. The 0.1 g of contaminated parts were placed in petri dishes containing PDA (as mentioned above). The growing fungus on PDA were identified macroscopically and microscopically according to the procedure above. Its inhibiting ability against oyster mushroom was also tested referring to the formula as mentioned above.

RESULTS AND DISCUSSION

Prevalence of air–borne and baglog–borne fungus. The spores captured using Petri dishes with 5 replications were 24±2 cfu/petri dish. Air–borne fungus were cuptured *Aspergillus* sp. 10 isolates, *Aspergillus* 7 isolates *niger, Brachysporium* sp. 1 isolate, *Cunninghamella* sp. 1 isolate, *Fusarium* sp. 19 isolates, *Geotrichum* sp 1 isolate, *Neurospora* sp. 8 isolates, *Mucor* spp. 18 isolates., *Penicillium* sp. 2 isolates, *Umbelopsis* sp. 1 isolate, *Stachybotrys* sp. 5 isolates, and *Trichoderma* sp. 1 isolate (Table 1, Fig. 1). The highest prevalence was dominated by *Fusarium* sp. with isolates frequency of 25.7%, followed by *Mucor* spp. 24.3%, *Aspergillus* spp. 13.5%, *Neurospora* spp. 10.8%, *Aspergillus niger* 9.5%, *Stachybotrys* spp. 6.7%, while the other was 1.4% (Table 1).

Based on Table 1, 7 of the 10 species of baglog–borne fungus were also air–borne, this means that 70% of fungal contaminants were derived from air–borne fungus and the rest (30%) originated from the baglog substrate. Fungal contaminants from contaminated baglog were dominated by *Trichoderma* spp. with the highest prevalence (35.71%), followed by *Aspergillus* spp. (21.43%), *Penicillium* spp. (14.28%), *Neurospora* spp.

Table 1. Number of air–borne, baglog–borne fungus, and their prevalence

No.	Fungus	Air–borne fungus		Baglog–borne fungus	
		Number of isolates	Prevalence (%)	Number of isolates	Prevalence (%)
1.	*Aspergillus* spp.	10	13.5	9	21.43
2.	*A. niger*	7	9.4	0	0
3.	*Brachysporium* sp.	1	1.4	0	0
4.	*Cunninghamella* sp.	1	1.4	0	0
5.	*Fusarium* spp.	19	25.6	1	2.38
6.	*Geotrichum* sp.	1	1.4	0	0
7.	*Gliocladium* sp.	0	0	1	2.38
8.	*Mucor* spp.	18	24.3	2	4.76
9.	*Neurospora* spp.	8	10.8	4	9.52
10.	*Paecilomyces* sp.	0	0	1	2.38
11.	*Penicillium* spp.	2	2.7	6	14.28
12.	*Pythium* sp.	0	0	1	2.38
13.	*Stachybotrys* spp.	5	6.7	2	4.76
14.	*Trichoderma* spp.	1	1.4	15	35.71
15.	*Umbelopsis* sp.	1	1.4	0	0
		74		42	

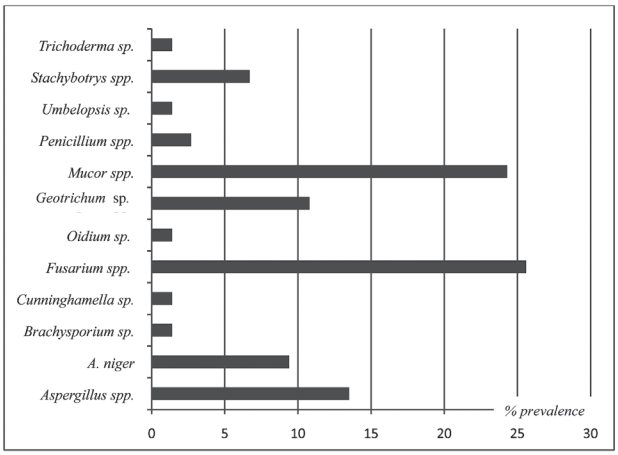

Fig. 1. Prevalence of air–borne fungus may could potentially to be fungal contaminants.

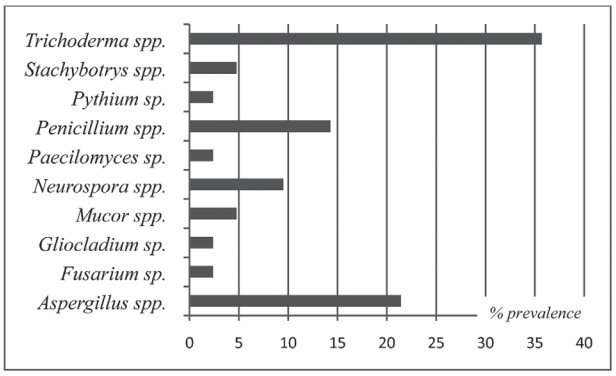

Fig. 2. Prevalence of fungal contaminants derived from baglog.

(9.52%), *Mucor* spp. (4.76%), *Fusarium* sp. (2.38%), *Gliocladium* sp. (2.38%), and *Paecilomyces* sp. (2.38%) (Table 1 and Fig. 2).

Trichoderma spp. which thrive on compost media (materials baglog) was the most virulent species with inhibiting mechanism was space and nutrient competition, mycoparasitic, and antibiosis. Cellulolytic fungi such as *Aspergillus*, *Penicillium*, and *Trichoderma* associated with the cellulose degradation were able to speed up the composting process for efficiency. This fungus may cause disease in oyster mushroom known as green mold disease (Sharma *et al*., 2007). Omokaro and Ogechi (2013) stated that fungus isolated from oyster mushroom substrate with its prevalence is *Aspergillus* (40.9%), *Fusarium* (22.7%), *Mucor* (5.6%), *Penicillium* (17,0%), *Rhizopus* (11.6%) and *Trichoderma* (2.3%). Likewise, Wickremasinghe *et al*. (1999) in Sri Lanka, also isolated and identified fungus from compost substrate such as *Aspergillus fumigatus*, *Chaetomium thermophile*, *Mucor pusillus*, and *Trichoderma harzianum*.

Trichoderma spp. attacks its host, establishing a structure resembling papilla and grow freely on its host hypha. The structure may form glycerol as a transcription of genes involved in lipid catabolism and then increase osmoregulation during contact to the host in mycoparasitism mechanism process (Druzhinina *et al*., 2011) .

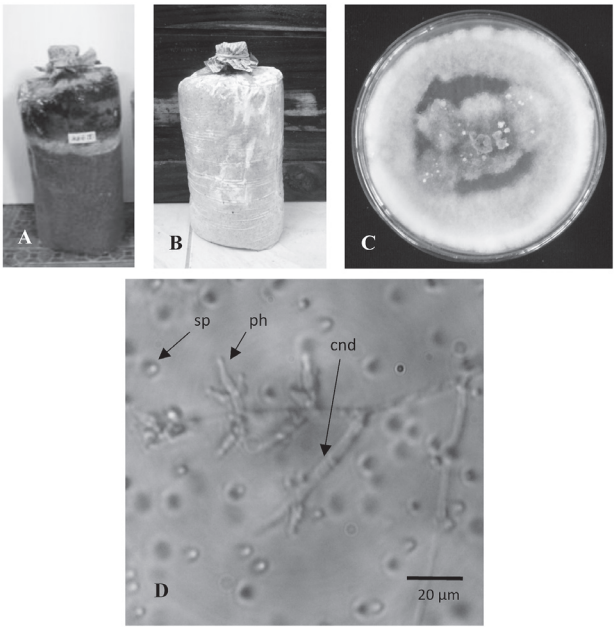

Fig. 3. Symptoms of baglog contaminated by *Trichoderma* sp. (A), Non–contaminated baglog (B), *Trichoderma* sp. colony in Petri dish (C), and Fruiting body of *Trichoderma* sp. 400x (D), (sp=spore, ph=phialide, and cnd=conidiophore).

Symptoms on contaminated baglog of *Trichoderma* sp. was green that gradually creeping into black. Symptoms begin to appear at the top of baglog and evolve towards the bottom. This means that the contamination was caused by air–borne fungi when seed planting into baglog. Contaminated baglog has dark colored compared to bright brown on noncontaminated baglog. *Trichoderma* hypha showed branched with conidiophores and phialide (Fig. 3).

Inhibiting ability of air–borne and baglog–borne fungi

The highest inhibiting ability of air–borne fungus against oyster was showed by *Mucor* spp. with inhibition of 94.7±8.5%, followed by *Aspergillus* niger (93.95± 9.7%) and *Stachybotrys* spp. (92.59±1.51%) (Table 2).

While the highest inhibiting ability of baglog contaminated fungus was *Mucor* spp. (91.11±12.57%), followed by *Trichoderma* spp. (84.96±7.36%), *Penicillium* spp. (84.33±5:02), *Pythium* sp. (83.33±0.1%) etc. (Table 2). According to the table, all air–borne and most of baglog–borne fungus showed inhibiting ability more than 75%. These means that the contaminated fungus were very significant to decrease the quality and quantity of oyster production.

The inhibition mechanism of air–borne fungus against oyster almost entirely showed a competition (competition of space and nutrients) except *Trichoderma* sp. was found an antibiosis and competition as well. While the inhibition mechanism of fungal contaminants originating from baglog almost all exhibited antibiosis mechanism, except *Stachybotrys* spp. that showed competition of space and nutrients.

Antibiosis mechanism of the fungus against oyster

Table 2. Percent inhibition of air–borne and baglog–borne fungus

No.	Fungus	Percent inhibition	
		Air–borne fungus	Baglog–borne fungus
1.	*Aspergillus* spp.	83.88±4.78	80.97±8.17
2.	*Aspergillus niger*	93.95±9.72	0
3.	*Brachysporium* sp.	0	0
4.	*Cunninghamella* sp.	0	0
5.	*Fusarium* spp.	85.14±9.6	72.22±0.2
6.	*Geotrichum* sp.	88.89±0.1	0
7.	*Gliocladium* sp.	0	70±0.1
8.	*Neurospora* sp.	91.67±9.14	82.22±1.7
9.	*Mucor* spp.	94.7±8.5	91.11±12.57
10.	*Paecilospora* sp.	0	82.22±0.2
11.	*Penicillium* spp.	77.78±0.1	84.33±5.02
12.	*Pythium* sp.	0	83.33±0.1
13	*Umbelopsis* sp.	77.78±0.1	0
14.	*Stachybotrys* spp.	92.59±1.51	78.71±9.71
15.	*Trichoderma* sp.	88.89±0.2	84.96±7.36

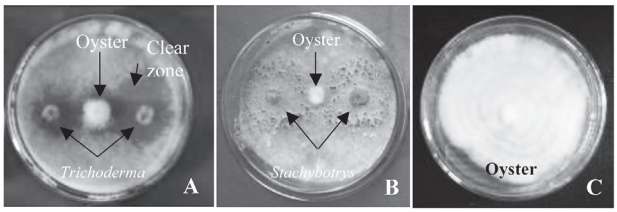

Fig. 4. Antibiosis mechanism between *Trichoderma* and oyster (A), Competition between *Stachybotrys* spp. and oyster (B), and oyster fungus (C).

was exhibited by existing of clear zone between two opposit colony in petri dish. Between the two colonies probably existed antibiotic substance (not verified) which came out from contaminants fungus as showed in Fig. 4.

Ashraf *et al*. (2007) found that fungus associated with oyster mushroom growing medium (compost) were *Aspergillus*, *Trichoderma*, *Mucor*, *Penicillium*, *Alternaria*, *Cladosporium*, *Monilia*, *Helminthosporium*, *Coccidioides*, and *Scedosporium*. This means that most the air–borne spores were potentially harm oyster mushrooms cultivation. Sharma *et al*. (2007) proved by their research in India that the fungus *Aspergillus* spp., Aspergillus niger, *Fusarium* spp., *Mucor* spp., and *Trichoderma* spp. acted as competitor fungus and caused disease in cultivated mushrooms such as oyster mushrooms. Lopez–Arevalo *et al*. (1996) also found that *Penicillium* sp., *Aspergillus* sp., *Trichoderma* sp., and *Cunninghamella* sp. were fungal contaminant in mushroom cultivation at tropical country especially Mexico.

Air–borne fungus could potentially cause failure of oyster mushroom cultivation. The highest prevalence fungi was *Fusarium* spp., while the highest inhibition was *Mucor* spp. Fungal contaminants originating from baglog with the highest prevalence was *Trichoderma* spp. This fungus was very dangerous to oyster mushroom cultivation.

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