International Journal of Agricultural Technology 2023 Vol. 19(1):203-214
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

The relationship between mycelial growth and fruit body's yield of oyster mushrooms (*Pleurotus* spp.) collected from southern Vietnam

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Pham, V. L., Pham, N. D. H., Nguyen, H. L. N., Nguyen, T. M. D., Nguyen, T. M, T., Nguyen, M. T., Nguyen, H. D. and Ho, B. T. Q. (2023). The relationship between mycelial growth and fruit body's yield of oyster mushrooms (*Pleurotus* spp.) collected from southern Vietnam. International Journal of Agricultural Technology 19(1):203-214.

Abstract *Pleurotus* spp. is one of the most important cultivated mushrooms in the world. The relationship between mycelial growth and production yield of *Pleurotus* strains collected from southern Viet Nam was determined.Total 10 of both wild and cultivated strains including 6 phoenix and 4 oyster strains were used in this study. The mycelial growth rate was conducted by 4 models such as the acreage of mycelial colonies on potato dextrose agar, the dried weight of mycelia in potato dextrose broth, the area of mycelial colonies on sawdust plates and the depth of mycelial colonies on sawdust testubes. The volume of mycelial colonies was estimated based on the area of mycelial colonies and their depth on sawdust plates. The results showed that the higher yield was obtained from wild mushroom strains. It was noted that there was a relationship between biological efficiency and volume of mycelial colonies of *P. pulmonarius*. These results provide the fundamentals for further studies on development of fast screening methods for *strains* of phoenix mushroom with high yield.

Keywords: Biological efficiency, *P. pulmonarius, P. ostreatus*, PDA, PDB, volume

Introduction

Pleurotus mushroom was the most important cultivated mushrooms in the world with the production ranked second after *Agaricus bisporus* and accounted for 19% of the total world production of cultivated mushrooms (Royse *et al.*, 2017). Some *Pleurotus* species cultivated in large scale are *P. ostreatus* complex, *P. cornucopiae, P. pulmonarius* complex, *P. tuber-regium*, *P. citrinopileatus* and *P. flabellatus* (OECD, 2006). The fruit bodies of

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Pleurotus mushroom had the high nutritional value, therapeutic properties (Cohen *et al*., 2002). In Vietnam, *Pleurotus* mushrooms were commonly grown, especially at southern region. Due to the ease of cultivation and high demand, the most cultivated species of *Pleurotus* were oyster mushroom (*P. ostreatus* complex) and phoenix mushroom (*P. pulmonarius* complex) (Tran *et al.*, 2017).

In Vietnam, mushroom strains could be collected in the wild, isolated from commercial products or originated from research institutes. Therefore, these strains are different in genetic, physiochemical properties and productivity. One of the issues in mushroom cultivation is that mushroom strains occasionally reduce yield after several consecutive subcultures or after a long period of storage in culture medium. The process of cultivating oyster mushrooms includes six main steps: selection of an acceptable mushroom, selection of a fruiting culture, development of spawn, preparation of compost, mycelial (spawn) running and mushroom development (Chang and Miles, 2004). One of the factors influencing cultivation time is the propagation of pure culture. Therefore, it is important to select the best fungal strains for the suitable mycelium growth rate and mycelium morphology.

Generally, the growth media for mushrooms contain carbon (C) and nitrogen (N) sources and potato dextrose agar (PDA) is generally known as the most common medium for growing fungi. However, many other media have been used for spawn development (Mahadevan and Shanmugasundaram, 2018; Nasim *et al*., 2001). Besides agar media, liquid media have also been used to culture spawn (Gapiński *et al*., 2007; Kupradit *et al*., 2020).

The evaluation of mycelial growth rate on agar media and biomass on liquid media is considered as the initial screening step to assess the quality of the spawn. Strains of high mycelial growth on PDA medium are usually selected for cultivation. Besides, mycelial growth rate on different substrates sush as sawdust, sugarcane bagasse... could also be crucial in evaluation of spawn quality. In Vietnam, many mushroom species are commonly cultivated on rubber sawdust. Therefore, evaluating growth rate of the mycelium on this subtrate could be an important way to select a good spawn and relationship between growth rates and yield must be studied extensively. So far, however, there has been no discussion on these relationships that could be found in the literature. In the present study, the mycelial growth of 10 wild and commercial strains of *Pleurotus* mushrooms collected from southern Vietnam was investigated. The biological efficiency (BE) of these strains and the relationship between the growth rate and BE was also evaluated to provide insights into this research area.

Materials and methods

Fungal strains

Ten strains of phoenix and oyster mushroom were obtained from Institute of Applied Biotechnogy, Ho Chi Minh City, Vietnam and maintained in malt yeast agar medium. These strains are wild and commercial strains which were collected at 7 provinces/cities in the Southern Vietnam. According to morphology and molecular properties, these mushroom strains belong to *P. pulmonarius* and *P. ostreatus* species. The details and particulars of the strains were presented in Table 1.

Table 1. Details of *Pleurotus* strains in this sudy

No.	Strain code	Species	Origin of strains (Province/city)	Commercial/ wild strains	Types
1	ABI-F000201	*P. ostreatus*	Ho Chi Minh	Commercial	Blue oyster
2	ABI-F000219	*P. ostreatus*	Can Tho	Commercial	Oyster
3	ABI-F000222	*P. ostreatus*	Ho Chi Minh	Commercial	Oyster
4	ABI-F000224	*P. ostreatus*	Dong Nai	Commercial	Oyster
5	ABI-F000241	*P. pulmonarius*	Vinh Long	Commercial	Phoenix
6	ABI-F000252	*P. pulmonarius*	Dong Nai	Commercial	Phoenix
7	ABI-F000253	*P. pulmonarius*	Dong Nai	Commercial	Phoenix
8	ABI-F000256	*P. pulmonarius*	Tay Ninh	Commercial	Phoenix
9	ABI-F000259	*P. pulmonarius*	Binh Thuan	Commercial	Phoenix
10	ABI-F000261	*P. pulmonarius*	Lam Dong	Wild	Phoenix

Study of the mycelial growth rate on PDA medium

Small pieces of vegetative cells (5 mm^2) obtained from the 7-day-old PDA slant were cited in the centre of petri dishes (9 cm diameter) containing 20 mL of sterile PDA medium (potato extract: 4 g/L, dextrose: 20 g/L, agar: 15 g/L). The mycelium discs were incubated at 25^oC in the dark. The area of the hyphal expansion was measured after 7 days of incubation using the ImageJ program (National Institutes of Health, USA). The average growth rate was calculated as follows:

$$\text{Growth rate } (mm^2/day) = \frac{\text{Mycelium area after 7 days of incubation (mm2)}}{\text{Incubation period (7 days)}}$$

Study of the production of fungal biomass on potato dextrose broth (PDB) medium

Small pieces of vegetative cells (5 mm^2) obtained from the 7-day-old PDA slant were transferred to 50 mL flasks containing 20 mL of sterile PDB

medium (potato extract: 4 g/L, dextrose: 20 g/L). The cultures were incubated at 25°C in static conditions for 7 days. The obtained mycelium was dried at 60 ℃ until constant weight.

Study of the mycelial growth rate on sawdust plates

Small pieces of vegetative cells (80 mm^2) obtained from the 7-day-old PDA slant were cited in the centre of petri dishes (9 cm diameter). Each petri dish contained 15 g of sterilized rubber sawdust. The moisture content of sawdust was adjusted to 65% before sterilization. The mycelium discs were incubated at 25°C in the dark. The area of the hyphal expansion was measured after 6 days of incubation using the ImageJ program and the average mycelial growth rate was determined.

Study of the mycelial running rate on sawdust test tubes

Small pieces of vegetative cells (80 mm^2) obtained from the 7-day-old PDA slant were cited in test tubes (25 mm diameter). Test tubes were filled and compressed with sawdust (28 g/150 mm) and enclosed with silicone rubber plugs. The moisture content of sawdust was adjusted to 65% before sterilization. The mycelium tubes were incubated at 25°C in the dark. The mycelial running rate was calculated by dividing the height of substrate in the test tubes (150 mm) by the duration for mycelia colonization and expressed as mm/day.

Fruiting body production

Oyster mushroom strains were cultured on PDA medium, and the mycelia were transferred to spawn medium (rice 100 g, rice bran 2 g, $CaSO_4.2H_2O$ 1 g, $CaCO_3$ 1 g, all were boiled together in 30 min). Fruiting medium (rubber sawdust 79 g, corn powder 20 g, $CaSO_4.2H_2O$ 1 g and moisture was controlled at 65%) were packed into polypropylene bags (size 20 × 12 cm, 1200 g substrates/bag). Substrates were sterilized and inoculated with spawn. Then the fully colonised bags were transferred into fruiting room and watering for fruiting body production. Finally, mushrooms were harvested when the mushroom cap surface was flat to slightly uprolled at the cap margins. Total weight of all the fresh fruiting bodies harvested from all the three pickings were measured and the biological efficiency (BE) was calculated as follows:

$$\text{BE (\%)} = \frac{\text{Total weight of harvested fresh mushroom per bag (g)}}{\text{Weight of dry substrate (g)}} \text{ x } 100 \text{ (Stamets, 2011)}$$

Experimental design and data analysis

The current experiments were arranged in completely randomized design with 5 replications except the fruiting body production was investigated using 50 bags per strain. Statistical analysis was conducted using StatDirect ver. 3.3 (StatDirect Ltd., Merseyside, UK). Differences between the means of individual groups were assessed by one-way ANOVA, Duncan's Multiple Range Test ($p<0.05$).

Results

The mycelial growth rate on PDA medium, sawdust plates, sawdust test tubes and fungal biomass on PDB medium

In this study, the effects of strains on the mycelial growth rate were investigated. The results showed that the mycelial growth rate on PDA medium of 10 mushroom strains were different and specific for each mushroom species. The highest growth rate was recorded with ABI-F000261 strain (wild strain), while ABI-F000201 resulted in the lowest growth rate (Table 2 and Figure 1).

Table 2. Growth rates and biomass production of *Pleurotus* strains on PDA medium, PDB medium, sawdust plates and sawdust test tubes

No.	Strain code	Growth rate on PDA (mm2/day)	Biomass dry weight on PDB (g/L)	Growth rate on sawdust plates (mm2/day)	Growth rate on sawdust test tubes (mm/day)
1	ABI-F000201	184.5g ± 19.4	3.14bcd ± 0.49	629.8e ± 98.4	5.69d ± 0.24
2	ABI-F000219	800.1b ± 43.8	3.37bc ± 0.32	681.1de ± 75.5	7.21b ± 0.16
3	ABI-F000222	512.7d ± 43.6	2.03ef ± 0.42	768.1bc ± 44.0	7.04bc ± 0.49
4	ABI-F000224	414.9e ± 56.3	2.95cd ± 0.81	729.1cd ± 45.5	7.11b ± 0.53
5	ABI-F000241	752.8bc ± 47.6	2.65de ± 0.43	819.2ab ± 20.8	7.08b ± 0.28
6	ABI-F000252	726.8c ± 22.7	2.25e ± 0.40	857.7a ± 43.0	7.66a ± 0.21
7	ABI-F000253	284.9f ± 39.5	1.41f ± 0.12	781.3abc ± 78.4	7.21b ± 0.16
8	ABI-F000256	369.6e ± 33.7	1.49f ± 1.10	722.5cd ± 58.8	6.64c ± 0.16
9	ABI-F000259	773.7bc ± 39.5	4.16a ± 0.32	716.4cd ± 23.2	7.67a ± 0.45
10	ABI-F000261	888.6a ± 45.5	3.73ab ± 0.41	841.2ab ± 27.9	7.81a ± 0.17

* All data were illustrated as mean ± standard deviation; Means (each column) followed by the same letters are not significantly different ($p<0.05$) by Duncan's multiple-range test; The alphabet lower case letters set were separated in each column.

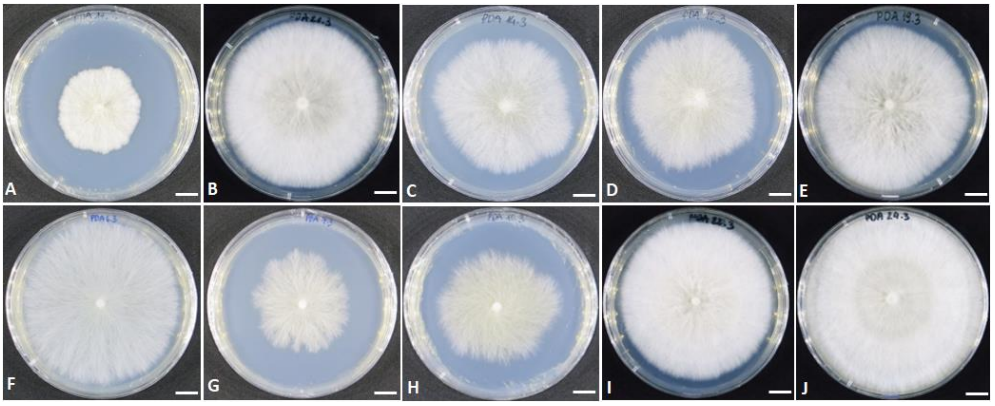

Figure 1. Colony morphology of *Pleurotus* strains on PDA medium after 7 days of incubation (A: ABI-F000201; B: ABI-F000219; C: ABI-F000222; D: ABI-F000224; E: ABI-F000241; F: ABI-F000252; G: ABI-F000253; H: ABI-F000256; I: ABI-F000259; J: ABI-F000261; bar: 1 cm)

In PDB, the mycelial biomass yield of *Pleutotus* strains was significantly different and specific for each mushroom species. The strains with the highest and the lowest biomass yield were ABI-F000259, ABI-F000261 (wild strain) and ABI-F000253, ABI-F000256, respectively (Table 2 and Figure 2).

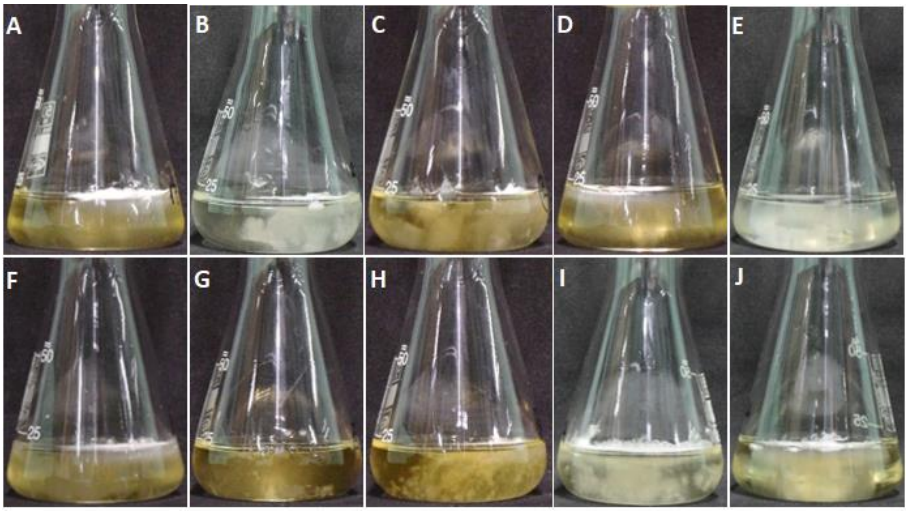

Figure 2. Biomass of *Pleurotus* trains on PDB medium after 7 days of incubation
(A: ABI-F000201; B: ABI-F000219; C: ABI-F000222; D: ABI-F000224; E: ABI-F000241; F: ABI-F000252; G: ABI-F000253; H: ABI-F000256; I: ABI-F000259; J: ABI-F000261)

In addition, 10 strains of *P. ostreatus* and *P. pulmonarius* were observed to grow and colonize on sawdust plates. The data revealed that the highest growth rate on sawdust plates of *Pleurotus* (857.7 mm^2/day) were found in case of ABI-F000252 strain. Strains with the lowest growth rates were ABI-F000201 and ABI-F000219 (629.8 and 681.1 mm^2/day, respectively) (Table 2 and Figure 3).

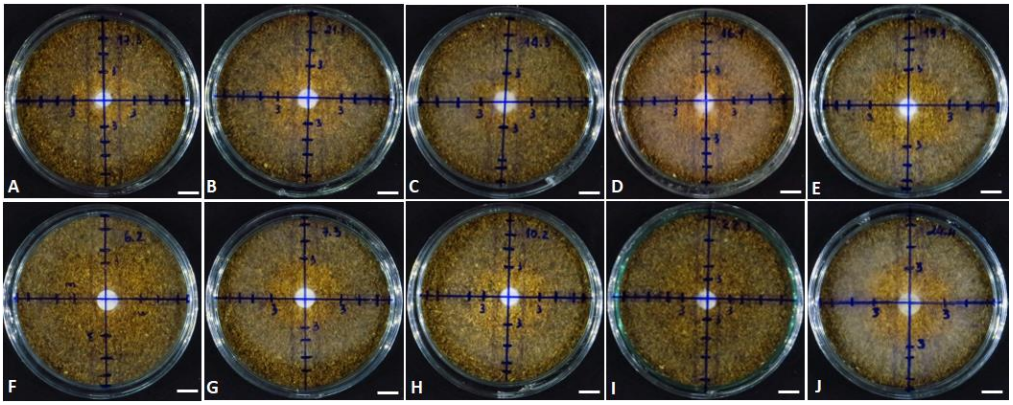

Figure 3. Colony morphology of *Pleurotus* strains on sawdust plates after 6 days of incubation (A: ABI-F000201; B: ABI-F000219; C: ABI-F000222; D: ABI-F000224; E: ABI-F000241; F: ABI-F000252; G: ABI-F000253; H: ABI-F000256; I: ABI-F000259; J: ABI-F000261; bar: 1 cm)

On the sawdust test tubes, it was also noticed that the mycelial running rates of *Pleutotus* strains were significantly varied between the investigated mushroom species. The strains with the highest and the lowest mycelial running rates were ABI-F000261 (wild strain) and ABI-F000201, respectively (Table 2 and Figure 4).

Biological efficiency and volume of mycelial colonies

In the present study, 10 mushroom strains were divided into two groups. Group 1 consisted of 4 fungal strains belong to *P. ostreatus* species (ABI-F000201 - ABI-F000224) and group 2 consisted of 6 fungal strains belong to *P. pulmonarius* species (ABI-F000241 - ABI-F000261).

The biological efficiency and volume of mycelial colonies of *P. ostreatus* and *P. pulmonarius* strains were presented in Table 3 and Table 4, respectively.

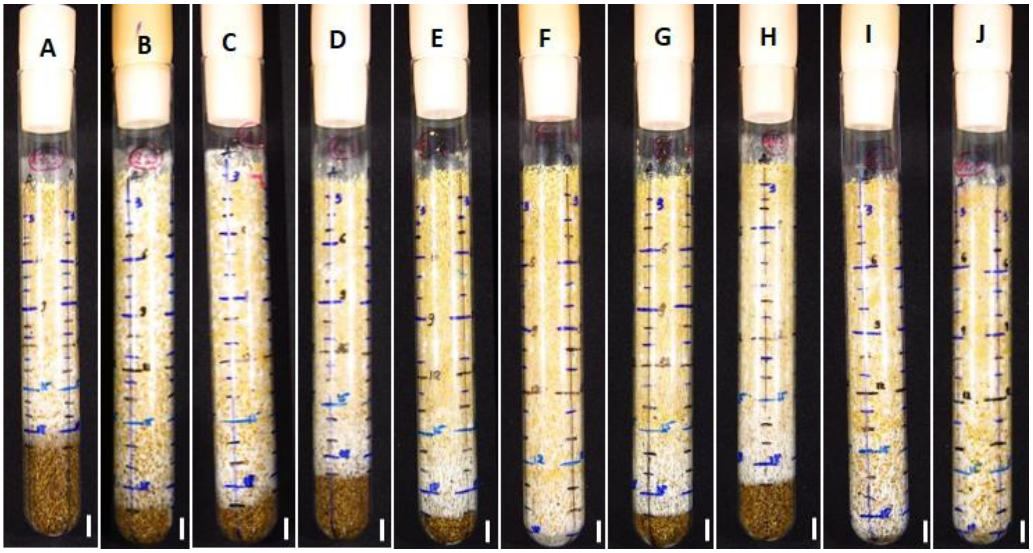

Figure 4. Mycelia of *Pleurotus* strains on sawdust test tubes after 19 days of incubation (A: ABI-F000201; B: ABI-F000219; C: ABI-F000222; D: ABI-F000224; E: ABI-F000241; F: ABI-F000252; G: ABI-F000253; H: ABI-F000256; I: ABI-F000259; J: ABI-F000261; bar: 1 cm)

Table 3. The biological efficiency and volume of mycelial colonies of *P. ostreatus* strains

No.	Strain code	Biological efficiency (%)	Volume of mycelial colonies
1	ABI-F000201	38.03c ± 4.55	3597.17b ± 648.19
2	ABI-F000219	46.52b ± 3.92	4910.34a ± 533.00
3	ABI-F000222	46.05b ± 5.63	5403.13a ± 472.53
4	ABI-F000224	49.73a ± 5.78	5185.73a ± 556.21

* All data were illustrated as mean ± standard deviation; Means (each column) followed by the same letters are not significantly different ($p<0.05$) by Duncan's multiple-range test; The alphabet lower case letters set were separated in each column.

Table 4. The biological efficiency and volume of mycelial colonies of *P. pulmonarius* strains

No.	Strain code	Biological efficiency (%)	Volume of mycelial colonies
1	ABI-F000241	19.22b ± 0.76	5806.38b ± 342.00
2	ABI-F000252	22.34a ± 2.06	6572.75a ± 471.48
3	ABI-F000253	17.96b ± 3.35	5625.64b ± 467.25
4	ABI-F000256	16.02c ± 3.70	4801.10c ± 459.50
5	ABI-F000259	14.29d ± 3.35	5493.18b ± 314.71
6	ABI-F000261	23.43a ± 3.38	6570.30a ± 235.89

* All data were illustrated as mean ± standard deviation; Means (each column) followed by the same letters are not significantly different ($p<0.05$) by Duncan's multiple-range test; The alphabet lower case letters set were separated in each column.

The results in Table 3 confirmed that the biological efficiency of *P. ostreatus* was significantly different among the cultivated strains. The BE of ABI-F000224 was highest at 49.73%, followed by ABI-F000219, ABI-F000222 of which the BE was in the range of 46.05% - 46.52%. The lowest BE (38.03%) was observed in case of ABI-F000201 strain. Meanwhile, the volume of mycelial colonies of ABI-F000219, ABI-F000222 and ABI-F000224 was similar (in the range of 4910.34 - 5403.13 mm^3) and higher than that of ABI-F000201 (3597.17 mm^3). There was no relationship between BE and volume of mycelial colonies of *P. ostreatus.*

For *P. pulmonarius*, the data in Table 4 also showed significantly different BE was observed among the cultivated strains. The BE of ABI-F000261 was highest at 23.43%, followed by ABI-F000252, ABI-F000241, ABI-F000253, ABI-F000256 of which the BE was in the range of 16.02% - 23.34%. The lowest BE (14.29%) was observed in case of ABI-F000259 strain. On the other hand, the volume of mycelia colonies of ABI-F000261 and ABI-F000252 was the highest and the lowest volume was obtained in case of ABI-F000259. Therefore, the volume of mycelical colonies of *P. pulmonarius* could be grouped into 3 categories including high volume (ABI-F000261, ABI-F000252), medium volume (ABI-F000241, ABI-F000253) and low volume (ABI-F000256, ABI-F000259). Especially, the strains in these groups of volume were the same with those in the decreasing order of BE, therefore, a strong relationship between volume and BE was observed in this study.

Discussion

PDA is the most popular medium for mycelial growth of the cultivated mushrooms. In this study, the mycelial growth rates of different strains were different for each species. By subculturing or preservation, quality of mycelium strains was changed. In this study, the mycelial growth rates on PDA medium of all the commecial strains were less than that of the wild strain. It could be due to the degeneration of oyster mushrooms by subculturing. The growth rates of *P. pulmonarius* on PDA medium were observed to be in range of 284.9 - 888.6 mm^2/day, which was higher than those reported by Stanley and Nyenke (2011) (114.83 mm^2/day) and Ilyas and Avin (2018) (217.11 mm^2/day).

For *P. ostreatus* strains, the growth rates were in the range of 184.5-800.1 (mm^2/day), which was similar to the data in previous reports. Hoa and Wang (2015) reported that the average mycelium diameter of *P. ostreatus* species was 9 cm at 8 days after inoculation (908.43 mm^2/day). Nguyen and Ranamukhaarachchi (2020) showed that the colony diameter of *P. ostreatus* on PDA medium was 6.43 cm after 7 days of incubation (465.13 mm^2/day), while

it was 4.2 cm after 7 days of incubation (199.71 mm^2/day) in the study of Kupradit *et al.* (2020).

On PDB medium, the mycelium biomass yields of *Plerotus* strains were significantly different after 7 days of incubation. The data revealed that strains with low growth rate on PDA medium, also resulted in low biomass yields on PDB medium. The biomass yields in this study were lower than the result of 3.76 g/L reported by Kupradit *et al.* (2020). This might be explained by the variations in the temperatures in culture condition in their research and our study (30 ^oC and 25^oC). Barakat and Sadik (2014) reported that mycelium biomass of *Pleurotus ostreatus* on Mushroom Complete Medium using different sugar types were 0.98 g/L – 5.46 g/L after 10 days of incubation.

On the sawdust plates, the growth rates of the strains were fairly high (629.8 – 857.7 mm^2/day). Even though the growth rates of some strains were low on PDA medium, the growth rates of these strains on sawdust substrate were high (ABI-F000201, ABI-F000253). The mycelial growth rates of ABI-F000252 and ABI-F000261 were the highest among the strains investigated in this study.

On the sawdust test tubes, the mycelial running rate of *Pleurotus* strains was significantly different. Substrate tubes displayed full growth within 19-26 days of inoculation. These results were in accordance to findings of Shah *et al.* (2008), Girmay *et al*. (2016), Adewoyin and Ayandele (2018). They reported that the spawn running took 17.33 - 21.33 days and the highest rate (7.81 mm/day) was also observed for the wild strain. In this study, the running rates of *P. pulmonarius* species were 6.64 – 7.81 (mm/day), while those of *P. ostreatus* species were 5.69 – 7.21 (mm/day). Ilyas and Avin (2018) reported that the mycelial running rate of *P. pulmonarius* strain was 5.24 cm after 8 days of incubation (6.55 mm/day). This result is quite similar to data reported by Miah *et al.* (2017), showing that the mycelial running rate of *P. ostreatus* was 6.3 – 7.6 mm/day.

Biological efficiency is a widely used parameter to evaluate the efficiency of substrate conversion in mushroom cultivation. In this study, the biological efficiency varied significantly among the different cultivated strains. Specifically, a wide range of BE (13.52 - 23.43%) was observed for *P. pulmonarius* strains, however these BE were lower than those reported by Adebayo *et al*. (2018) (54%), Adewoyin and Ayandele (2018) (25.56% - 36.13%). In the meanwhile, a higher BE range (38.03 - 49.73%) was obtained in case of *P. ostreatus* strains but it was far lower than that reported by Shah *et al.* (2004) (64.69%) (Shah *et al*., 2004), Bhattacharjya *et al*. (2014) (187 - 213.2%) On the other hand, Girmay *et al*. (2016) found a lower BE of *P. ostreatus* (9.73%) compared to our results.

In conclusion, mycelial growth rates and BE of ten oyster mushroom strains were observed to be significantly different and specific for each mushroom species. Of all the strains tested, ABI-F000252 was the most compatible with Vietnam's climate (high mycelial growth rate, high biological efficiency) for commercial *P. pulmonarius* trains while ABI-F000224 was best strain for *P. ostreatus* species. Wild trains (ABI-F000261) resulted in high biological efficiency and mycelium growth rates. Therefore, this strain has the potential to be domesticated. The results from the present study also showed the possibility to array and select good spawn based on volume of mycelial colonies.

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(Received: 15 September 2022, accepted: 30 December 2022)