# Improvement of propagation media in abalone mushroom (*Pleurotus abalonus*) cultivation

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Abstract The eight different media were tested for mycelium growth capacity.-The modified PDA medium supplemented with either 0.4% yeast extract (MT1), or 0.4% yeast extract and 0.5% peptone (MT3), was found to be suitable for mycelial propagation. In addition, MT3 medium was found to be superior as it did not affect successive mycelial growth on seed medium. Furthermore, among the 4 testing different culture media, G2 medium with a mixture of 95.0% rubber sawdust, 5.0% rice husk, 3.0% corn powder, 1.0% sugar, 1.0% CaCO<sub>3</sub>, 0.03% NH4Cl, 0.03% MgSO<sub>4</sub>, and 0.03% KH<sub>2</sub>PO<sub>4</sub> was found to be the most suitable compost for spawn running and had a biological efficiency of  $39.62\pm3.89\%$ . In summary, a propagation medium and a culture compost for abalone mushroom cultivation are identified. An alternative compost culture with 90% rubber sawdust and 10.0% rice husk is proposed to ensure the rational use of rubber sawdust resources in the future.

Keywords: Abalone mushroom, Compost culture, Mushroom cultivation, *Pleurotus abalonus*, Propagating medium

# Introduction

*Pleurotus* spp. are among the most cultivated and commercialized edible mushrooms in the world because of their delecious taste, high nutritional value, and other valuable biological properties (Golak-Siwulska *et al.*, 2018; Raman *et al.*, 2021; Sánchez, 2010). Among those, *Pleurotus abalonus*, also known as abalone mushroom, in addition to the nutritional value, has also been shown to have a number of biological activities, such as antioxidant, anti-proliferative, and immunomodulatory activities on U937 leukemia cells of the ethanolic and aquaeous extracts (Panthong *et al.*, 2016; Wongjaikam *et al.*, 2019). Studies on the bioactive chemical composition of *P. abalonus* fruiting body have revealed a number of potential health-promoting compounds. Polysaccharide compounds from *P. abalonus* fruit bodies have shown high antioxidant activity and inhibited

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the reproduction of human colorectal carcinoma cells (Ren et al., 2015). The polysaccharide-peptide complex LB-1b from P. abalonus has also been shown to have antioxidant, hypoglycemic, and inhibitory activities against the liver cancer cell lines HepG2 and breast cancer cells MCF7 (Li et al., 2012). Several compounds include 9-beta-d-ribofuranosidoadenine (ADO), 5-deoxy-5-(methylthio) adenosine (MTA), and triterpenoid complexes have been shown to inhibit hemolysis, lipid peroxidation in the brain and kidneys, and scavenge free hydroxyl radicals (Zhang et al., 2013). In addition, the polysaccharide LA from the fruiting body of *P. abalonus* has also been shown to exhibit inhibitory activity on HIV-1 reverse transcriptase activity (Wang *et al.*, 2011). Overall, the available research suggests that *P. abalonus* is a promising source of bioactive compounds with a wide range of potential health benefits. However, more research is needed to improve the production of this species since it has been much less cultivated and studied compared to other oyster mushrooms such as P. ostreatus, P. florida, and P. cystidiosus (Raman et al., 2021; Sánchez, 2010).

Up to date, only a few studies have been carried out to investigate the cultivation condition of *Pleurotus abalonus* such as effects of media compositions, temperature, initial pH, moisture content, different extracts of sapwood and heartwood on mycelial growth; as well as fruit body production using sawdust from various wood species (Ohga, 2000; Takayama *et al.*, 1993). In Vietnam, the cultivation of *Pleurotus abalonus* mushrooms is currently similar to the cultivation process of other oyster mushrooms using rubber sawdust as the main substrate. However, the current cultivation process results in slow mycelial growth at all propagation levels, high risk of pathogen infection, limited development of fruit body and reduced yield. Therefore, the aim of this study was to investigate the culture media and cultivation compost components, as well as to shorten the cultivation time with high biological efficiency.

#### Materials and methods

#### Fungal strain and spawn preparation

*Pleurotus abalonus* was isolated from fruiting bodies provided by Trang Sinh Mushroom Company (38B/2 Hoc Lac, Ward 14, District 05, Ho Chi Minh City) on PDA medium (potato dextrose agar), at room temperature (30±2°C). Spawn tubers were continuously inoculated on PDA and stored at 4°C for further experiments.

# Pleurotus abalonus mycelia growth on various media formulations

Eight media used to test the growth of *P. abalonus* mycelium were listed in Table 1. Agar and broth media were prepared for the growth of *P. abalonus* spawn. Agar media (20 g L<sup>-1</sup>) was prepared in Petri dishes (12 mL per dish) and broth media (50 mL per bottle) was prepared in glass bottles. Spawn was propagated on PDA medium and inoculated into the Petri dishes and glass bottles with 5 mm × 5 mm mycelia pieces. Petri dishes and glass bottles were incubated at room temperature ( $30\pm2^{\circ}C$ ) and monitored for mycelium growth. Mycelium growth on agar plates was assessed by measuring the diameter of the mycelial growth in days until the entire plate was covered. The density of mycelium on agar plates was observed by optical microscopy at x40 magnification. For broth media, the dry biomass of mycelium was determined after 20 days of incubation.

 Table 1. Agar media for P. abalonus mycelial propagation

Medium	Composition (per Liter)					
PDA	200g potato infusion, D-glucose 20.0g					
MT1	200g potato infusion, D-glucose 20.0g, yeast extract 4.0g					
MT2	200g potato infusion, yeast extract 4.0g, peptone 5.0g					
MT3	200g potato infusion, D-glucose 20.0g, yeast extract 4.0g, peptone 5.0g					
Agaricus	200g potato infusion, D-glucose 20.0g, peptone 2.0g, MgSO4.7H2O 0.5g,					
	Na <sub>2</sub> HPO <sub>4</sub> 2.0g					
SDAY1	<i>D</i> -glucose 40.0g, yeast extract 2.0g, peptone 10.0g					
SDAY2	D-glucose 40.0g, yeast extract 2.0g, peptone 10.0g, MgSO <sub>4</sub> .7H <sub>2</sub> O 0.5g, KH <sub>2</sub> PO <sub>4</sub>					
	1.0g					
Raper	D-glucose 20.0g, yeast extract 2.0g, peptone 2.0g, MgSO4.7H2O 0.5g, KH2PO4					
	$0.5g, K_2HPO_4 \ 1.0g$					

# Investigation of mushroom mycelia growth on spawn grains

The ability of *P. abalonus* mycelia to grow on spawn seed was tested. Paddy was boiled until its shell cracked and then 5.0% rice bran, 0.1% urea, and 0.5% yeast extract were added. The ingredients were mixed, distributed into glass bottles, and autoclaved at 121°C for 60 minutes. After sterilization, the bottles were inoculated with *P. abalonus* mycelia on the best nutrient agar and incubated at room temperature. The length of the mycelium was measured in both directions of the glass bottles every day.

#### Investigation of mushroom mycelia growth on compost mixtures

Four compost mixtures of rubber sawdust and rice husk were prepared according to the following formulas: G1: 100% sawdust; G2: 95% sawdust + 5%

rice husk; G3: 90% sawdust + 10% rice husk; G4: 85% sawdust + 15% rice husk. Rice husk and sawdust were incubated with a 1.0% lime solution for 24 hours, and nutrients were added (3.0% corn powder, 1.0% sugar, 1.0% CaCO<sub>3</sub>, 0.03% NH<sub>4</sub>Cl, 0.03% MgSO<sub>4</sub>, and 0.03% KH<sub>2</sub>PO<sub>4</sub>) and the humidity was adjusted to 60-65% (Akter *et al.*, 2022; Hoa & Wang, 2015; Takayama *et al.*, 1993; Wang *et al.*, 2012). The mixtures were packed into polypropylene bags (20x30 cm) with a final weight of 600 grams and were sterilized at 121°C for 2 hours. The bags were inoculated with spawn seeds and incubated at room temperature ( $30\pm2^{\circ}$ C). The spawn running on compost mixtures was determined according to the length of mycelia from day to day until the compost was completely covered.

#### Biological efficiency of Pleurotus abalonus on various composts

After the bags were completely covered with mycelium, they were transferred to culture rooms with a temperature of 25-30°C and a relative humidity of 85-90%. The humidity was maintained by sprinkling water on the floor and walls of the culture rooms several times a day. Mushroom fruiting bodies were harvested in the first flush, and the biological efficiency (BE) was calculated to confirm the yield of each compost as following: BE (%) = (weight of fresh mushrooms/weight of dry substrate) x 100.

#### Data analysis

Experiments were conducted with three replicates for tests of mycelia growth on various nutrient agar media and mushroom spawn seed. The tests on various composts were repeated on 15 packets. The collected data were expressed as the mean±standard deviation. All data were processed using Microsoft Excel 365 and statistics were done using Statgraphics Centurion XVIII (One-way ANOVA) software with a P < 0.05 was considered statistically significant.

# Results

# Effect of various nutrient media on Pleurotus abalonus mycelial growth

No mycelial growth was observed on any of the eight tested media until the  $3^{rd}$  day of incubation, when mycelia began to grow and well attach to the surface of all tested media. The mycelia continued to grow over time, and after 20 days of incubation, the diameter of the mycelia on MT1 and MT3 media was the greatest, ranging from  $6.33\pm0.20$  to  $6.90\pm0.17$  cm. The diameter of the mycelia

on Raper, PDA, and SDAY1 media was slower, ranging from  $5.27\pm0.17$  to  $5.77\pm0.17$  cm. The mycelial diameter on MT2, SDAY2, and Agaricus media was the slowest, with a diameter of  $3.57\pm0.17$  to  $3.67\pm0.27$  cm (Table 2).

**Table 2.** Mycelial growth in time course of *Pleurotus abalonus* on different nutrient agar media

Media	Colony diameter (cm)						
	5 days	8 days	11 days	14 days	17 days	20 days	
PDA	$1.60{\pm}0.20$	2.10±0.17	2.77±0.27	3.43±0.17	4.67±0.17	5.77±0.17	
SDAY1	$1.57 \pm 0.17$	$2.10{\pm}0.20$	$2.93 \pm 0.27$	$3.57 \pm 0.20$	$4.57 \pm 0.07$	5.27±0.17	
MT3	2.17±0.13	$3.07 \pm 0.17$	3.83±0.17	4.77±0.17	5.77±0.17	6.90±0.17	
MT1	$1.90{\pm}0.17$	2.73±0.13	$3.57 \pm 0.17$	4.33±0.13	$5.27 \pm 0.07$	$6.33 \pm 0.20$	
Raper	$1.93{\pm}0.07$	2.67±0.13	$3.40{\pm}0.07$	4.17±0.13	4.90±0.17	5.57±0.23	
MT2	$1.90{\pm}0.17$	$2.43 \pm 0.27$	2.77±0.17	$3.33 \pm 0.27$	3.33±0.17	3.57±0.17	
Agaricus	$1.90{\pm}0.17$	$2.10{\pm}0.07$	$2.27 \pm 0.07$	$2.57{\pm}0.07$	3.10±0.17	3.67±0.27	
SDAY2	$1.90{\pm}0.17$	2.10±0.17	2.23±0.17	$2.57 \pm 0.17$	$3.10{\pm}0.07$	3.67±0.17	

Potato dextrose agar=PDA; medium trial=MT; Sabouraud dextrose agar yeast=SDAY.



**Figure 1.** Macroscopic growth of *P. abalonus* on various nutrient agar media after 20 days of incubation. Potato dextrose agar=PDA; medium trial=MT; Sabouraud dextrose agar yeast=SDAY

The structure of the mycelial network also varied among the different media, especially after 20 days of incubation. The mycelia grew evenly, densely, and close to the agar surface in MT1, MT3, Raper, and PDA media. The density

and evenness of the mycelia was reduced in SDAY1, SDAY2, MT2, and Agaricus media (Figure 1).



**Figure 2.** Microscopically observed mycelium density (A); alphabetical order of letters indicates *P. abalonus* mycelia on PDA, SDAY1, MT3, Agaricus, MT1, SDAY2, Raper, and MT2 media, respectively under light microscope at x40 magnification. Mycelium biomass in different media after 20 days of incubation (B); Column displays mean value of three replicates and bar displays the standard deviation. Means with different letters above columns statistically differ from each other (P < 0.05). Potato dextrose agar=PDA; medium trial=MT; Sabouraud dextrose agar yeast=SDAY

The density of the mycelium was further examined at x40 magnification, and the results were shown in Figure 2A. The density of the mycelium on MT3 and MT1 media was denser and tighter than that of the mycelium on PDA medium. The mycelial network of *P. abalonus* on Agaricus, Raper, and SDAY2 media had a sparse structure with a thin mycelial density. Meanwhile, the mycelium on SDAY1 medium was winding and irregularly branched, with a tangled mycelial network structure. In addition, the biomass of *P. abalonus* mycelium cultured in the 8 corresponding broths was collected after 20 days of incubation and the dry weight was determined (Figure 2B). The most biomass of the mycelium was collected in MT1 and MT3 media, with values of  $0.562\pm0.068$  g and  $0.608\pm0.037$  g, respectively. Potato dextrose agar medium gave a lower biomass (~1.4-1.52 folds,  $0.40\pm0.005$  g) than MT1 and MT3 media but was higher than the other media. The biomass formed in the remaining media was similar, ranging from  $0.323\pm0.032$  g to  $0.368\pm0.023$  g. Therefore, *P. abalonus* mycelium was best developed on MT3 and MT1 media in terms of mycelial spread, biomass, and mycelial density. Compared with the PDA medium commonly used in mushroom spawn propagation, the *P. abalonus* mycelium grown on MT1 and MT3 media was higher than PDA in terms of mycelial spread rate (~25%) and biomass (~35%).

#### Pleurotus abalonus mycelial running in grain spawn

Propagation of mushroom spawn on grains is a common practice in mushroom cultivation in order to rapidly increase the biomass of mycelium (UN.ESCAP, 2008). However, continuous inoculation in nutrient-rich media may reduce the mycelia biosynthesis of extracellular hydrolytic enzymes. In this experiment, the ability of *P. abalonus* mycelia to grow on grain media was tested to confirm the quality of mushroom mycelia cultured on MT1 and MT3 nutrient-rich agar media. Equal amounts of mycelia on MT1 and MT3 nutrient agar were inoculated into prepared grain bottles (GMT1 and GMT3, respectively) and their growth at different time intervals was observed and recorded. The mycelia GMT1 and GMT3 showed clear signs of development and well-expended in all directions with faster growth was observed in GMT3 (Figure 3A).



**Figure 3.** Growth of *P. abalonus* mycelia on grain spawn inoculated with mycelia from MT1 medium (A-a) and MT3 medium (A-b): Growth in time course of mycelia in different grain spawns (B), grain spawn from MT medium=GMT, day after incubation=DAI. Column displays mean value of three replicates and bar displays the standard deviation. Different letters above columns indicate statistically significant differences between two grain media in various incubation time (P < 0.05)

After 10 days of incubation, the migration length of mycelium in GMT3 was highest compared to GMT1 with 1.3 folds, followed by 1.22 folds different after 14 days. The mycelium covered the entire grain spawn of GMT3 ( $6.27\pm0.35$  cm) with a large, dense, and strong mycelial network after 20 days of incubation but was slower ( $5.43\pm0.23$  cm) in GMT1 (Figure 3B).

#### Pleurotus abalonus growth on various composts

The ability of mycelium to run on substrates is a basis for evaluating the quality of mushroom spawn, which affects the incubation time, yield, and economic efficiency of fruiting body production. In this study, rubber sawdust was used as the main substrate as it is the most commonly used in mushroom cultivation in Vietnam. Mycelial running of *P. abalonus* was tested on various compost mixtures of rubber sawdust and rice husk in different proportions.

The results showed that *P. abalonus* mycelia developed with the lowest in G4 compost mixture of 85% sawdust and 15% rice husk, and the best growth in G2 compost mixture of 95% sawdust and 5% husk. There was no difference of mycelia running on G1 and G3 compost mixtures (Figure 4). After 10 days of incubation, growth of *P. abalonus* on all 4 compost mixtures was observed. However, mycelia running on G4 compost explored discrete mycelial network, thin mycelium, and almost no growth was observed for longer periods of incubation time after 40 days. *Pleurotus abalonus* mycelial growth on G2 compost with 5% rice husk showed the best results. The time to complete the mycelial running in spawn packets was 40 days, which was shorter than the time required for G1 and G3 compost with 5% rice husk.



**Figure 4.** *Pleurotus abalonus* mycelial migration in time course (A) and Growth in compost packets after 35 and 45 days of incubation (B): Day after incubation=DAI. G1 = 100% rubber sawdust, G2 = 95% sawdust + 5% rice husk, G3 = 90% sawdust + 10% rice husk, G4 = 85% sawdust + 15% rice husk. Line shows mean value of fifteen replicates and bar shows the standard deviation

# Biological efficiency of P. abalonus on various composts

The biological efficiency of different compost mixtures was evaluated on the yield in the first flush of fruiting bodies. After the *P. abalonus* mycelia cover all entirely packets, these packets were moved into the mushroom culture room with temperature conditions of 25-30°C and high humidity of culture room was maintained at 85-90%. Packets containing G4 compost mixture were not moved into the culture room because the *P. abalonus* mycelia was weak and did not cover the bags after 50 days of incubation time.





**Figure 5**. *Pleurotus abalonus* fruiting bodies on spawn packets (A) and biological efficiency of *P. abalonus* on various composts (B): G1 = 100% rubber sawdust, G2 = 95% sawdust + 5% rice husk, G3 = 90% sawdust + 10% rice husk. Column performs mean value of three replicates and bar shows the standard deviation. Different letters above columns indicate statistically significant differences between three compost media in various incubation time means (*P*<0.05)

After 4 days, packets containing G2 compost mixture started to make pin forms and produce fruiting bodies, while packets containing G1 and G3 compost mixture made pin forms after 5 days of watering in culture room. Mature fruiting bodies were collected, and fresh weight determined (Figure 5A). The biological efficiency of *P. abalonus* on G2 compost mixture (39.62 $\pm$ 3.89%) was determined to be significantly higher than the biological G2 compost mixture on

two G1 ( $28.7\pm 4.43\%$ ) and G3 compost mixture ( $22.95\pm 4.34\%$ ) (Figure 5B). G3 compost mixture had complete mycelium running time in packets similarly as control G1 compost mixture, however the biological yield was noted to be lower. Statistical analysis results showed that there was no significant difference in biological efficiency between these two compost mixtures.

### Discussion

Abalone mushrooms are popularly cultivated in many countries. However, the slow growth of *P. abalonus* mycelia in propagation media increases the cost of mushroom cultivation. Improvement of the composition of the spawn media for *P. abalonus* mushroom is necessary for the development of this fungus cultivation. In this study, the spawn medium and compost for cultivating P. abalonus were improved and obtained high efficiency. Spawn medium was modified from PDA control medium added with 0.4% yeast extract and 0.5% peptone (MT3). PDA is the control medium and the simplest and most commonly used medium in the current mushroom cultivation technology (UN.ESCAP, 2008). On PDA medium, mycelium length, density, and biomass all gave satisfactory results, but only after MT1 and MT3. The superior growth of P. abalonus mycelium on MT1 and MT3 media compared to PDA and the others could be explained by their nutrient compositions. The MT1 and MT3 media contain potato extract, glucose, and yeast extract, which provide protein, carbohydrates, vitamins, and minerals essential for mycelium growth. The higher in mycelium length and density as well as slightly higher biomass obtained on MT3 than on MT1 may be due to the presence of additional peptone in MT3 medium. Peptone is an organic nitrogen source containing amino acids and short peptides, which can be metabolized easily and rapidly by the mushroom mycelium, and thus promote better mycelium growth. In addition, statistical analysis showed that *P. abalonus* mycelium on MT2 medium, which lacks glucose components, resulted in lower mycelium growth than on MT1 and MT3 media. However, high glucose concentration in SDAY1 and SDAY2 media did not help to rapidly increase the growth of mycelium since these two media do not contain potato extract, which provides a mixture of carbohydrates, minerals, proteins, vitamins, and other growth promoting factors. As a result, it could be deduced that potato extract and glucose are the core components for mycelia growing. In addition, propagation of mushroom spawn on grains is a common practice in mushroom cultivation in order to rapidly increase the biomass of mycelium, P. abalonus mycelium on MT3 medium showed good growth and development on grains. The results showed that MT3 including richer in nutrition did not affect the production of extracellular hydrolytic enzymes by the mushroom mycelia and was in favor of efficient and shortened spawn production

time. Therefore, MT3 medium was proposed as propagation media of P. *abalonus*.

The cultivation mushroom compost is also improved with the addition of 5% rice husk to the sawdust substrate. The compost mixture containing 5% rice husk (G2 compost mixture) is suitable for the cultivation of P. abalonus with the result of shortening time to complete mycelium running by 8 days compared to the control G1 compost with only rubber sawdust as the substrate. It was confirmed that G2 compost mixture required only 4 days from stimulation to primordial initiation and was faster than other compost mixtures. In addition, the biological efficiency is  $\sim 10\%$  higher than others in the first flush. This is the first study on mixing rice husk into rubber sawdust substrate to grow P. abalonus. The similar results were also observed in studies on mixing rice husk into the substrate to cultured fungi P. ostreatus with a biological yield of 64% after 4 harvests (addition of 5% rice husk) (Frimpong-Manso et al., 2011), or 56.5% when 25% rice husk was mixed with sawdust to grow *P. ostreatus* (Akter *et al.*, 2022). In addition, rice husk added to the P. tuberregium culture substrate also resulted in increased yield and the amount of ash, fat, and carbohydrate in the mushroom fruiting bodies (Okigbo et al., 2021). Shortening the incubation and growing time will save energy costs, fast production rotation, reduce related costs, and achieve higher economic efficiency for the production of *P. abalonus*. In addition, the similarity of mycelium running time and biological efficiency between the control compost G1 with 100% rubber sawdust and the G3 compost mixture supplemented with 10% husk also suggested a mixing method to reduce the need for use of rubber sawdust sources. This accounts for a high proportion of costs in mushroom cultivation and the rice husk supplementary into mushroom compost also supports sustainability in the rational use of resources. In overall, the obtained results, therefore, facilitate the growth of *P. abalonus* mushrooms and develop sustainability in the use of mushroom growing substrates.

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