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ESTABLISHING THE STOCK CULTURES COLLECTION OF PROPECTIVE *Pleurotus* MUSHROOMS

SUMMARY OF DISSERTATION ON BIOTECHNOLOGY

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INTRODUCTION

1. The necessity of the thesis

In Vietnam, approximately 50 million tonnes of agricultural waste were generated annually, presenting an opportunity for the profitable commercialization of mushroom cultivation. However, the mushroom industry's growth was limited both production and number of cultivated species. To develop mushroom cultivation in Vietnam, it is crucial to establish climate-resilient and high-yielding varieties. Nonetheless, mushroom varieties originated from various sources, with most sourced from foreign countries and only a small number collected from domestic sources. Information regarding varieties, farming productivity, and spawn quality was either lacking or inconsistent, with spawn quality frequently being unstable over time. Mycelia for mushroom production were typically maintained in subsequent cultures using solid media and often exhibit symptoms of strain degeneration. Therefore, specialized equipment is necessary to preserve and develop techniques for preserving and restoring varieties, with a focus on breeding and improving varieties.

Pleurotus mushroom was the most important cultivated mushrooms in the world with the production ranked second after *Lentinula edodes* (Royse *et al*., 2017). The fruit bodies of *Pleurotus* mushroom had the high nutritional value, therapeutic properties. In Vietnam, *Pleurotus* mushrooms were commonly grown, especially in the southeast 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*). However, the quality of spawn

was not paid attention so it should be focused on strains improvement to drive production efforts forward.

Based on practical considerations, the establishing of the stock cultures collection of *Pleurotus* mushrooms based on information of the origin, identification, genetic diversity and characteristics, which are currently being commonly produced and consumed in the market, would establish a fundamental basis for subsequent applied research.

2. The research objectives

Establish the stock cultures collection of dikaryon strains and monokaryon isolates of commonly commercial oyster mushroom and phoenix mushroom.

3. The main research contents

- Collect and identify commonly cultivated *Pleurotus* mushroom strains.
- Investigate some biological characteristics of the collected *Pleurotus* mushroom strains.
- Collect and investigate some biological characteristics of monokaryon isolates of *Pleurotus* mushroom.
- Investigate the ability to recognize mating types of monokaryon isolates of some molecular biological markers.

Chapter 1. OVERVIEW

1.1. INTRODUCTION ABOUT OYSTER MUSHROOM

1.1.1. Overview

Oyster mushroom (*Pleurotus* spp.) is one of the most distributed edible mushrooms worldwide with the production ranked second after *Lentinula edodes*. *Pleurotus* is considered as a delicacy with high nutritional and medicinal values and has diverse biotechnological

applications. With over 25 recognized species cultivated around the world, *Pleurotus* is also one of the diverse groups of cultivated edible mushrooms. This genus is distributed in a wide range of tropical and temperate regions. Until now, many problems in taxonomic classification and phylogenetic relationships of the *Pleurotus* species are not well understood. Singer (1986) recognized 36 species in the genus. According to Chang and Miles (2004) there were 50 valid species are recognized in the genus.

1.1.2. Life cycle and mating types

The life cycle of *Pleurotus* species includes two main phases: haploid monokaryotic phase (monokaryon - n) and a haploid dikaryotic phase (dikaryon - n + n) (Barh *et al*., 2019). The fusion of two compatible monokaryotic hyphae (clamp connection) results in the formation of a dikaryon. The formation of dikaryotic mycelium arises through the fusion of two monokaryons with different alleles in both A and B mating type loci (AxBx, AyBy).

1.2. COLLECT AND PRESERVE MUSHROOM STRAINS

1.2.1. Culture collection

Strains collections are obtained from various sources such as original strains sourced from research institutions and seed banks, commercial strains used in cultivation, and indigenous strains isolated from wild strains.

1.2.2. Culture preservation

- Short-term storage: periodic transfer.
- Long-term storage: starvation of nutrients, limitation of oxygen, lyophilization, freezing (Chang and Miles, 2004).

1.3. CLASSIFICATION OF OYSTER MUSHROOMS

The classification of oyster mushrooms is important because it supports various fields including conservation of genetic resources and biodiversity. This is the first step in the researches on characterization of mushrooms. One of the primary methods for accurate classification of oyster mushrooms is based on their macroscopic and microscopic characteristics. Besides, other methods are also used individually or in combination for species identification.

1.3.1. Classification of oyster mushrooms based on morphological characterization

Morphological analysis requires careful observation of features and comparison with taxonomic keys.

1.3.2. Classification of oyster mushrooms based on mating compatibility

The principle of this method is based on the hybridization between the mycelia of the unidentified species and the determined species of the genus *Pleurotus*. Species that are different from the compatible group will not be able to interbreed (Shnyreva and Shtaer, 2006).

1.3.3. Classification of oyster mushrooms based on consensus sequence

Most of the classification methods in this group use consensus sequences located in genes encoding ribosomal DNA (rDNA) and a few genes encoding some other specific proteins. Among the sequence regions, ITS is the most widely used sequence region in fungal genetics (Shnyreva *et al*., 2012).

1.4. AFLP ANALYSIS OF GENETIC DIVERSITY

AFLP (Amplified Fragment Length Polymorphism) technology has been widely employed in the analysis of genetic diversity in macro fungi. The AFLP technique was introduced by Vos *et al.* (1995) and has become a popular method for assessing genetic diversity. AFLP can rapidly evaluate genetic diversity via the selective amplification of DNA fragments cleaved by two restriction enzymes, followed by PCR.

1.5. EVALUATION OF SPAWN QUALITY

The evaluation of spawn quality is conducted at different levels: based on DNA and gene expression, enzymes of the fungi strain and biochemical tests, growth of the mycelium on different nutrient media. The most important is the evaluation based on the growth rate and biological efficiency (BE) in cultivation.

1.6. BREEDING TECHNIQUES FOR STRAINS IMPROVEMENT

Some common breeding objectives of oyster mushroom are higher yield, fruit body quality, sporelessness, resistance against abiotic and biotic stresses… Several breeding techniques for strains improvement in *Pleutotus* including mutation breeding, genetic transformation, protoplast fusion, mycelial mating. Among these, crossing between two monokaryotic mycelia is a prevalent and straightforward method for generating novel strains.

Chapter 2. MATERIALS – METHODS

2.1. COLLECT, IDENTIFY AND ANALYSIS GENETIC DIVERSITY COMMONLY CULTIVATED *Pleurotus* MUSHROOM STRAINS

2.1.1. Collect samples

Strains of oyster mushrooms and phoenix mushrooms from commercial sources in southern provinces were collected.

2.1.2. Sample treatment, strains isolation

After observation, description, photography, on-site isolation or isolation in the laboratory, the fruiting bodies of the mushrooms were sampled (Nguyen Lan Dung, 2014).

2.1.3. Morphological analysis

The morphological characteristics were described following Largent (1977) and Largent *et al.* (1977). The identification was followed Miller (1969), Corner (1981), Petersen and Krisai-Greilhuber (1996), Segedin *et al*. (1995), Petersen and Krisai-Greilhube (1999), Guzmán (2000), Lechner *et al*. (2005), Zmitrovich and Wasser (2016).

2.1.4. Phylogenetic analysis

Pleurotus mushroom samples were identified based on the ITS region sequence using the method described by James *et al.* (2006), and species phylogenetic trees were constructed using the MEGA X software.

2.1.5. Genetic diversity analysis based on AFLP

The genetic diversity among these strains was evaluated by amplified fragment length polymorphism (AFLP) markers, followed Pawlik *et al*. (2012).

2.2. BIOLOGICAL CHARACTERISTICS OF *Pleurotus* CULTIVARS

2.2.1. Study of the mycelial growth rate on PDA and PDB medium

2.2.1.1. Study of the mycelial growth rate on PDA medium

Cultivate Petri dishes until the mycelial network of the initial strain completely covers the plate, and determine the rate of mycelial growth (mm^2/day).

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

The experiment was done under static liquid conditions, as described by Kupradit et al. (2020), with a few modifications.

2.2.2. Study of the mycelial growth rate on sawdust

2.2.2.1. Study of the mycelial growth rate on sawdust Petri plates

Fungal inoculation was done on sawdust plates until the mycelial network of the initial strain completely colonized the plate, and determine the rate of mycelial growth (mm^2/day).

***2.2.2.2.* Study of the mycelial running rate on sawdust test tubes**

Cultivate fungal cultures in test tubes on sawdust substrate and determine the mycelial growth rate (mm/day).

2.2.3. Study of the decolorizing ratio

Study of the decolorizing ratio was done according to Magae *et al.* (2005) with a few modifications.

2.2.4. Study of biological efficiency and relationship between mycelial growth on sawdust and biological efficiency

2.2.4.1. Study of biological efficiency

Cultivate on a sterilized medium (sawdust 79%, corn bran 20%, $CaSO_4$ 1%) and determine the biological efficiency (Stamets, 2011).

2.2.4.2. Relationship between mycelial growth on sawdust and biological efficiency

2.3. MONOKARYOTIC CHARACTERISTICS OF *Pleurotus* CULTIVARS

2.3.1. Monokaryotic isolates collecting and preservation

4 *Pleurotus* strains were chosen to collect and determine monokaryotic isolates: ABI-F000241, ABI-F000252, ABI-F000253 and ABI-F000224 strains. Single basidiospore isolation was done according to Gharehaghaji *et al.* (2007) with a few modifications.

2.3.2. Study of the monokaryons growth rate on PDA medium

Perform a similar procedure as described in section 2.2.1.1.

2.3.3. Study of the monokaryons decolorizing ratio

Study of the decolorizing ratio was done according to Magae *et al.* (2005) with a few modifications.

2.3.4. Determination of mating types of monokaryons

Study of the mating types of monokaryons was done according to Tran Thi Ngoc My *et al.* (2005).

2.4. THE ABILITY TO RECOGNIZE MATING TYPES OF MONOKARYON ISOLATES OF SOME MOLECULAR BIOLOGICAL MARKERS

The study selected eight monokaryotic isolates, representing four mating types alleles of a phoenix strain.

2.4.1. Analysis genetic diversity of monokaryotic isolates based on AFLP

Perform a similar procedure as described in section 2.1.5.

2.4.2. Analysis the ability to recognize mating types of monokaryon isolates of some specific primers.

Chapter 3. RESULTS – DISCUSSIONS

3.1. COLLECT, IDENTIFY, ANALYSIS GENETIC DIVERSITY COMMONLY CULTIVATED *Pleurotus* MUSHROOM STRAINS

3.1.1. Collecting and preservation *Pleurotus* mushroom strains

In total, 15 strains of *Pleurotus* mushroom were collected from eight provinces/cities located in the southern region, including Dong Nai, Tay Ninh, Binh Duong, Ba Ria - Vung Tau, Ho Chi Minh City, Binh Thuan, Vinh Long, Can Tho and Lam Dong. All these strains, except for the wild strain from Lam Dong, were commercially sourced. Among them, ten strains belonged to the phoenix mushroom group, four strains to the oyster mushroom group, and one strain to the blue oyster group.

3.1.2. Morphological analysis

3.1.2.1. Phoenix mushroom cultivars

Many species's macroscopic and microscopic features were similar with *P. ostreatus* and *P. pulmonarius's* descriptions. But microscopic feature (spore length/width ratio - Q value: 2.1 – 2.5), cap shape and their pileipellis thick were similar with *P. pulmonarius's* description. So, according to morphology, 10 cultivars belong to *P. pulmonarius.*

3.1.2.2. Oyster mushroom cultivars

Many species's macroscopic and microscopic features were similar with *P. ostreatus* and *P. pulmonarius's* descriptions. But microscopic feature (Q value: 2.7 – 2.9), cap colour and their pileipellis thick were similar with *P. ostreatus's* description. So, according to morphology, 4 cultivars belong to *P. ostreatus.*

3.1.2.3. Blue oyster mushroom cultivar

ABI-F000201 strain's macroscopic and microscopic features were similar with *P. ostreatus* and *P. pulmonarius's* descriptions. But microscopic feature (Q value: 2.8) and their pileipellis thick were similar with *P. ostreatus's* description. So, according to morphology, this cultivar belongs to *P. ostreatus.*

3.1.3. Molecular analysis

3.1.3.1. ITS sequence analysis

Upon comparison with GenBank data, all mushroom strains were found to belong to the genus *Pleurotus* with high similarity. Specifically, 10 samples were identified as *P. pulmonarius*, while 5 samples were identified as *P. ostreatus*. The identification results based on ITS sequence were compatible with the morphological identifications above.

3.1.3.2. Phylogenetic analysis

The phoenix mushroom strains were identified as *P. pulmonarius* and formed a cluster with a bootstrap value of 88% together with 8 reference sequences of *P. pulmonarius*. Meanwhile, the oyster mushroom and blue oyster strains formed another cluster with a bootstrap value of 80% together with 5 reference sequences of the *Pleurotus* cf. *floridanus* group.

3.1.4. Genetic diversity analysis based on AFLP

The AFLP analysis results revealed high genetic diversity among the collected *Pleurotus* mushroom strains, with similarity score ranging from 44 to 88%. The 15 strains were distributed into 2 main clusters (Figure 3.2). Clusters 1 consisted of five *P. ostreatus* strains, with similarity score ranging from 72-84%. Meanwhile, clusters 2 consisted

of ten *P. pulmonarius* strains, with similarity score ranging from 71-88%. Strains belonging to the same species typically had a closer genetic relationship than other species.

Figure 3.1. Maximum likelihood (ML) tree based on ITS sequence

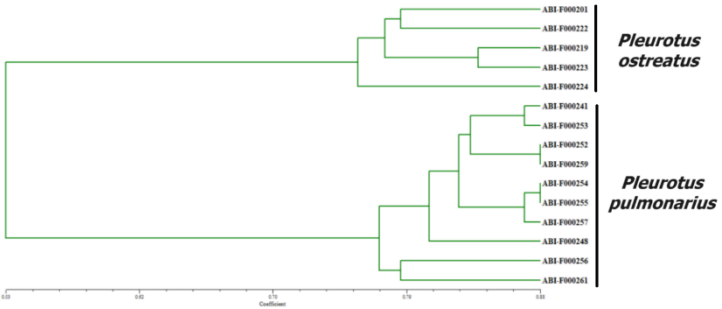

Figure 3.2. UPGMA tree based on the genetic diversity of the 15 *Pleurotus* mushroom strains using four AFLP markers.

3.2. BIOLOGICAL CHARACTERISTICS OF *Pleurotus* CULTIVARS

3.2.1. Study of the mycelial growth rate on PDA and fungal biomass on PDB medium

Table 3.1. Growth rates and biomass production of *Pleurotus* strains on PDA medium, PDB medium

No.	Strain code	Species	Growth rate on PDA (mm^2/day)	Biomass dry weight on PDB (g/L)
1	ABI-F000201	*P. ostreatus*	$184.5^{g} \pm 19.4$	$3.14^{bcd} \pm 0.49$
2	ABI-F000219	*P. ostreatus*	$800.1^{b} \pm 43.8$	$3.37^{bc} \pm 0.32$
3	ABI-F000222	*P. ostreatus*	$512.7^{d} \pm 43.6$	$2.03^{ef} \pm 0.42$
4	ABI-F000224	*P. ostreatus*	$414.9^{e} \pm 56.3$	$2.95^{cd} \pm 0.81$
5	ABI-F000241	*P. pulmonarius*	$752.8^{bc} \pm 47.6$	$2.65^{de} \pm 0.43$
6	ABI-F000252	*P. pulmonarius*	$726.8^{c} \pm 22.7$	$2.25^{e} \pm 0.40$
7	ABI-F000253	*P. pulmonarius*	$284.9^{f} \pm 39.5$	$1.41^{f} \pm 0.12$
8	ABI-F000256	*P. pulmonarius*	$369.6^{e} \pm 33.7$	$1.49^{f} \pm 1.10$
9	ABI-F000259	*P. pulmonarius*	$184.5^{g} \pm 19.4$	$3.14^{bcd} \pm 0.49$
10	ABI-F000261	*P. pulmonarius*	$800.1^{b} \pm 43.8$	$3.37^{bc} \pm 0.32$

The results showed that the mycelial growth rate on PDA medium of 10 mushroom strains were different and specific for each mushroom species (Table 3.1). The highest growth rate was recorded with ABI-F000261 strain (wild strain), while ABI-F000201 resulted in the lowest growth rate. In PDB, the mycelial biomass yield of *Pleutotus* strains was significantly different and specific for each mushroom species (Table 3.1).

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

The results showed that the mycelial growth rate on sawdust plates of 10 mushroom strains were different and specific for each mushroom species (Table 3.2). The data revealed that the highest growth rate on sawdust plates of *Pleurotus* were found in case of ABI-F000252 strain. Strains with the lowest growth rates was ABI-F000201 strain.

Table 3.2. Growth rates of *Pleurotus* strains on sawdust plates and sawdust test tubes

No.	Strain code	Species	Growth rate on sawdust plates (mm^2/day)	Growth rate on sawdust test tubes (mm/day)
1	ABI-F000201	*P. ostreatus*	$629.8^{e} \pm 98.4$	$5.69^{d} \pm 0.24$
2	ABI-F000219	*P. ostreatus*	$681.1^{de} \pm 75.5$	$7.21^{b} \pm 0.16$
3	ABI-F000222	*P. ostreatus*	$768.1^{bc} \pm 44.0$	$7.04^{bc} \pm 0.49$
4	ABI-F000224	*P. ostreatus*	$729.1^{cd} \pm 45.5$	$7.11^{b} \pm 0.53$
5	ABI-F000241	*P. pulmonarius*	$819.2^{ab} \pm 20.8$	$7.08^{b} \pm 0.28$
6	ABI-F000252	*P. pulmonarius*	$857.7^{a} \pm 43.0$	$7.66^{a} \pm 0.21$
7	ABI-F000253	*P. pulmonarius*	$781.3^{abc} \pm 78.4$	$7.21^{b} \pm 0.16$
8	ABI-F000256	*P. pulmonarius*	$722.5^{cd} \pm 58.8$	$6.64^{c} \pm 0.16$
9	ABI-F000259	*P. pulmonarius*	$716.4^{cd} \pm 23.2$	$7.67^{a} \pm 0.45$
10	ABI-F000261	*P. pulmonarius*	$841.2^{ab} \pm 27.9$	$7.81^{a} \pm 0.17$

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 (Table 3.2). The strains with the highest and the lowest mycelial running rates were ABI-F000261 (wild strain) and ABI-F000201, respectively.

3.2.3. Study of the decolorizing ratio

The decolorizing ratio on YBLB medium of strains was significantly different and specific for each mushroom strain (Table 3.3). The colour of media changed from green to yellow. The decolorizing ratio of strains ranged from 30.32% to 79.65%.

Table 3.3. Decolorizing ratio on YBLB medium of *Pleutotus* strains

No.	Strain code	Species	Decolorizing ratio (%)
1	ABI-F000201	*P. ostreatus*	$36.75^{cd} \pm 12.64$
2	ABI-F000219	*P. ostreatus*	$45.36^{bcd} \pm 15.15$
3	ABI-F000222	*P. ostreatus*	$53.40^{bc} \pm 7.85$
4	ABI-F000224	*P. ostreatus*	$51.79^{bc} \pm 13.60$
5	ABI-F000241	*P. pulmonarius*	$30.32^{d} \pm 20.90$
6	ABI-F000252	*P. pulmonarius*	$59.72^{b} \pm 11.84$
7	ABI-F000253	*P. pulmonarius*	$49.79^{bc} \pm 17.14$
8	ABI-F000256	*P. pulmonarius*	$79.65^{a} \pm 8.46$
9	ABI-F000259	*P. pulmonarius*	$45.64^{bcd} \pm 11.81$
10	ABI-F000261	*P. pulmonarius*	$39.48^{cd} \pm 12.19$

3.2.4. Study of biological efficiency and relationship between mycelial growth on sawdust and biological efficiency

The results in Table 3.4 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/day) and higher than that of ABI-F000201 (3597.17 mm^3/day).

Table 3.4. The biological efficiency and volume of mycelial colonies of *P. ostreatus* strains

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

Table 3.5. The biological efficiency and volume of mycelial colonies of *P. pulmonarius* strains

No.	Strain code	Biological efficiency (%)	Volume of mycelial colonies (mm^3/day)
1	ABI-F000241	$19.22^b \pm 0.76$	$5806.38^b \pm 342.00$
2	ABI-F000252	$22.34^a \pm 2.06$	$6572,75^a \pm 471.48$
3	ABI-F000253	$17.96^b \pm 3.35$	$5625,64^b \pm 467.25$
4	ABI-F000256	$16.02^c \pm 3.70$	$4801.10^c \pm 459.50$
5	ABI-F000259	$14.29^d \pm 3.35$	$5493.18^b \pm 314.71$
6	ABI-F000261	$23.43^a \pm 3.38$	$6570.30^a \pm 235.89$

For *P. pulmonarius*, the data in Table 3.5 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.

3.3. MONOKARYOTIC CHARACTERISTICS AND MATING TYPES OF *Pleurotus* CULTIVARS

3.3.1. Monokaryotic isolates *collecting and preservation*

3.3.1.1. Monokaryotic isolates collecting

The results showed that 80 monokaryotic isolates were collected (20 isolates per strain) and four types of monokaryon's colony morphology were observed: rooting type, cottony type, dense mycelial type and concentric striate type.

3.3.1.2. Monokaryotic isolates preservation

The monokaryotic isolates were cultured in MYA medium and preserved at 4 °C.

3.3.2. Mycelial growth rate on PDA medium of monokaryotic isolates

3.3.2.1. Monokaryotic isolates of ABI-F000241 strain

The mycelial growth rate on PDA medium of monokaryotic isolates were different (from 15.8 mm^2/day to 428.8 mm^2/day). The highest growth rate was recorded with No. 36 isolate, while No. 37 and No. 60 isolates resulted in the lowest growth rate.

3.3.2.2. Monokaryotic isolates of ABI-F000252 strain

The mycelial growth rate on PDA medium of monokaryotic isolates were different (from 26.9 mm^2/day to 399.2 mm^2/day). The highest growth rate was recorded with No. 15 isolate, while No. 16, 24, 30, 43 isolates resulted in the lowest growth rate.

3.3.2.3. Monokaryotic isolates of ABI-F000253 strain

The mycelial growth rate on PDA medium of monokaryotic isolates were different (from 72.5 mm^2/day to 410.8 mm^2/day). The highest growth rate was recorded with No. 23, 36, 44, 45 isolates, while No. 42, 54 isolates resulted in the lowest growth rate.

3.3.2.4. Monokaryotic isolates of ABI-F000224 strain

The mycelial growth rate on PDA medium of monokaryotic isolates were different (from 2.7 mm^2/day to 50.7 mm^2/day). The highest growth rate was recorded with No. 46 isolate, while No. 49 isolate resulted in the lowest growth rate.

3.3.3. Decolorizing ratio of monokaryotic isolates

3.3.3.1. Monokaryotic isolates of ABI-F000241 strain

The decolorizing ratio of isolates ranged from 11.23% - 89.54%. The highest rate was recorded with No. 45 isolate, while No. 36 isolate resulted in the lowest rate.

3.3.3.2. Monokaryotic isolates of ABI-F000252 strain

The decolorizing ratio of isolates ranged from 19.72% - 87.02%. The highest rate was recorded with No. 24 isolate, while No. 13 isolate resulted in the lowest rate.

3.3.3.3. Monokaryotic isolates of ABI-F000253 strain

The decolorizing ratio of isolates ranged from 14.29% - 86.97%. The highest rate was recorded with No. 08 isolate, while No. 47 isolate resulted in the lowest rate.

3.3.3.4. Monokaryotic isolates of ABI-F000224 strain

The decolorizing ratio of isolates ranged from 8.83% - 81.16%. The highest rate was recorded with No. 44 isolate, while No. 14 isolate resulted in the lowest rate.

The decolorizing ratio of monokaryotic isolates of mushroom strains was higher than oyter mushroom strains and parental dikaryon strains. The decolorizing ratio of most monokaryotic isolates was relatively high. Therefore, they could be used for breeding programs.

3.3.4. Determination of mating types of monokaryons

3.3.4.1. Determination of mating types for each strain

ABI-F000241 strain

ABI-F000241 strain had 3 monokaryotic isolates type A1B1 (01, 05, 08), 7 monokaryotic isolates type A2B2 (06, 09, 24, 33, 34, 43, 59), 5 monokaryotic isolates type A1B2 (04, 20, 26, 37, 60) and 5 monokaryotic isolates type A2B1 (13, 19, 23, 36, 45).

ABI-F000252 strain

ABI-F000252 strain had 8 monokaryotic isolates type A1B1 (02, 04, 12, 13, 15, 20, 22, 24), 5 monokaryotic isolates type A2B2 (16, 30, 34, 33, 36), 2 monokaryotic isolate type A1B2 (27, 29) and 5 monokaryotic isolate type A2B1 (07, 09, 31, 39, 43).

ABI-F000253 strain

ABI-F000253 strain had 5 monokaryotic isolates type A1B1 (04, 08, 09, 36, 54), 4 monokaryotic isolates types A2B2 (13, 27, 45; 51), 5 monokaryotic isolates type A1B2 (01, 20, 23, 24, 37) and 6 monokaryotic isolates type A2B1 (16, 41, 42, 44, 47, 52).

ABI-F000224 strain

ABI-F000224 strain had 2 monokaryotic isolates type A1B1 (20, 42), 7 monokaryotic isolates types A2B2 (14; 45; 46; 50; 60; 61; 64), 10 monokaryotic isolates type A1B2 (02; 05; 19; 35; 44; 47; 49; 54; 55; 62) and 1 monokaryotic isolate type A2B1 (18).

It was noted that *Pleurotus* species has a bifactorial tetrapolar mating system as other *Pleurotus* species.

3.3.4.2. Interstrain matings result of tester strains between three P. pulmonarius strains

As a result of these crosses, the numbers of A and B factors of three *P. pulmonarius* strains were 2 and 2, respectively. Because the numbers of A and B factors of the three strains were 2 and 2, these strains share the same origin.

3.4. ANALYSIS THE ABILITY TO RECOGNIZE MATING TYPES OF MONOKARYON ISOLATES OF SOME MOLECULAR BIOLOGICAL MARKERS

3.4.1. Analysis genetic diversity of monokaryotic isolates based on AFLP

The result showed that diversity of monokaryotic isolates was high with similarity coefficients ranging from 61 to 94%. Monokaryotic isolates with the same mating type allele showed a closer genetic relationship than those with different alleles. The phylogenetic tree model (Figure 3.3) divided the monokaryotic isolates into two clusters: clusters 1 consisted of isolates with the B1 mating type allele, while clusters 2 consisted of isolates with the B2 mating type allele. The AFLP results supported and confirmed the mating type determination above.

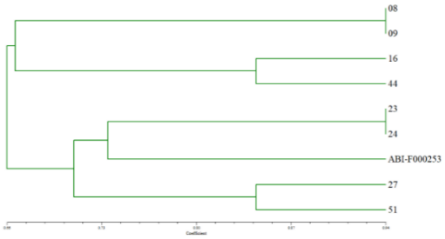

Figure 3.3. UPGMA tree based on the genetic diversity of the monokaryotic isolates using four AFLP markers.

3.4.2. Analysis the ability to recognize mating types of monokaryon isolates of some specific primers

3.4.2.1. Revalidation of the specificity of primer pairs on king oyster mushroom (P. eryngii)

The specificity of 10 primer pairs as published by Ju *et al*. (2020) was revalidated with a commercial king oyster mushroom strain. PCR products were obtained from 9/10 primer pairs (Figure 3.4). The specificity of the primer pairs was stable with king oyster mushroom.

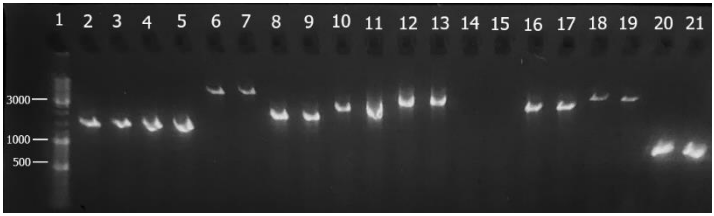

Figure 3.4. PCR product electrophoresis results of the 10 primer pairs on the king oyster mushroom

(*lane 1: DNA marker; lane 2, 3: pimer No. 1; lane 4, 5: primer No. 2; lane 6, 7: primer No. 3; lane 8, 9: primer No. 4; lane 10, 11: primer No. 5; lane 12, 13: primer No. 6; lane 14, 15: primer No. 7; lane 16, 17: primer No. 8; lane 18, 19: primer No. 9; lane 20, 21: primer No. 10*)

3.4.2.2. Assessment of specificity of 10 primer pairs with phoenix mushroom (P. pulmonarius) on bioinformatics data

The results showed that there were no suitable sequences for both forward and reverse primers analysis. If only analyzing the forward or reverse primers, there were no primers 100% matching sequences. Therefore, the primers used in *P. eryngii* were not specific enough to amplify target sequences in *P. pulmonarius*. The PCR test results on dikaryotic strain ABI-F000253 and the monokaryotic isolates showed similarity with the Blast results; not produce the desired electrophoresis bands for all of the used primer pairs. (Figure 3.5-3.8).

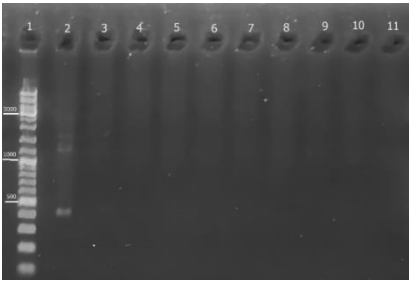

Figure 3.5. PCR product electrophoresis results of the primer pairs on the ABI-F000253 strain.

(*lane 1: DNA marker; lane 2: pimer No. 1; lane 3: primer No. 2; lane 4: primer No. 3; lane 5: primer No. 4; lane 6: primer No. 5; lane 7: primer No. 6; lane 8: primer No. 7; lane 9: primer No. 8; lane 10: primer No. 9; lane 11: primer No. 10)*

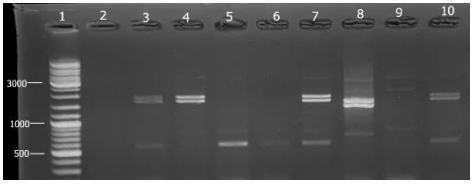

Figure 3.6. PCR product electrophoresis results of primer pair 1 at annealing temperature of 60°C on the monokaryotic isolates of the ABI-F000253 strain and the parental strain
(lane 1: DNA marker, lane 2: isolate 08, lane 3: isolate 09, lane 4: isolate 23, lane 5: isolate 24, lane 6: isolate 16, lane 7: isolate 44, lane 8: isolate 27, lane 9: isolate 51, lane 10: ABI-F000253 strain)

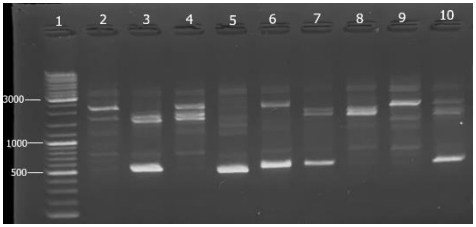

Figure 3.7. PCR product electrophoresis results of primer pair 1 at annealing temperature of 56°C on the monokaryotic isolates of the ABI-F000253 strain and the parental strain

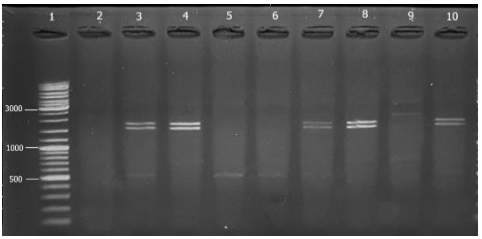

Figure 3.8. PCR product electrophoresis results of primer pair 1 at annealing temperature of 62°C on the monokaryotic isolates of the ABI-F000253 strain and the parental strain

Chapter 4. CONCLUSIONS AND SUGGESTIONS

4.1. CONCLUSIONS

- Established the stock cultures collection of propective dikaryon strains of *Pleutotus* mushroom from southern provinces. There were 10 strains of phoenix mushroom identified as *P. pulmonarius* species, 4 strains of oyster mushroom identified as *P. ostreatus* species, 1 strain of blue oyster mushroom identified as *P. ostreatus* species.
- Biological characteristics of *Pleurotus* muhroom strains have been determined. The strains in the collection have commercial potential. Of all the strains tested, ABI-F000252 was the most compatible with Vietnam's climate for commercial *P. pulmonarius* trains while ABI-F000224 was best strain for *P. ostreatus* species. Especially the wild mushroom strain ABI-F00261, which exhibited many favorable characteristics for cultivation.
- Established the stock cultures collection of 80 monokaryon isolates from 4 *Pleurotus* mushroom strains. The biological and mating characteristics of the monokaryotic isolates have been determined. The numbers of A and B factors of three *P. pulmonarius* strains were determined; and their shared genetic origin has been predicted.
- The AFLP data of the monokaryon isolates of phoenix mushroom strain ABI-F000253 were analyzed and the ability to group the isolates based on their mating type alleles was tested using several specific primer pairs.

4.2. SUGGESTIONS

- Increase the number of mushroom strains, modify the shape and size of the containers in the experiment to investigate the relationship between biological efficiency and growth rate on sawdust.
- Commercialization study of the wild strain ABI-F000261: nutritional composition evaluation, safety assessment...
- Collect commercial oyster mushroom samples from more distant geographical regions (central, northern areas), collect wild oyster mushroom samples to increase the diversity of alleles for determining mating types.
- Test to assess the ability to screen monokaryon isolates of phoenix mushroom using several specialized molecular biological markers.

NEW CONTRIBUTIONS OF THE THESIS

1. For the first time, relevant information on the morphology, biological characteristics, genetic characteristics, growth and development of 10 *Pleurotus* mushroom strains with commercial potential collected from the southern provinces has been provided.
2. Established a stock cultures collection of 80 monokaryon isolates from 4 *Pleurotus* mushroom strains, including information on mating types and growth characteristics.
3. Isolated and evaluated cultivation potential of a wild *Pleurotus* mushroom strain in Lam Dong province (ABI-F000261).

LIST OF THE PUBLICATIONS RELATED TO THE DISSERTATION

1. **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.

2. **Pham, V. L**., Pham, N. D. H., Nguyen, H. D., Le, T. H. and Ho, B. T. Q. (2023). Monokaryotic characteristics and mating types of phoenix mushroom (*Pleurotus pulmonarius*) cultivars in the south Vietnam. *International Journal of Agricultural Technology*, 19(1): 189-202.

3. **Pham Van Loc,** Ngo Thuy Tram, Le Thanh Nhan, Pham Nguyen Duc Hoang, Nguyen Hoang Dung, Ho Bao Thuy Quyen (2023). Identification of oyster mushroom (*Pleurotus* spp.) strains in the south Vietnam based on morphological characteristics and ITS sequencing. *Vietnam Journal of Agricultural Sciences*, 21(12): 1569-1580.