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Monokaryotic characteristics and mating types of phoenix mushroom (*Pleurotus pulmonarius*) cultivars in the South Vietnam

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Abstract Phoenix mushroom (*Pleurotus pulmonarius*) is one of the most important cultivated mushrooms in Vietnam. Monokaryon is a fundamental breeding material in conventional procedures of *Pleurotus* crossbreeding. In the present study, three *P. pulmonarius* strains originated from the southern of Vietnam were chosen to collect and determine monokaryotic isolates. Mycelial growth rate on agar medium and decolorizing rate of YBLB medium of these monokaryotic isolates were investigated. Then, mating types of monokaryons were determined by random hybridization. As a result, 60 monokaryotic isolates were collected and four types of monokaryon's colony morphology were observed including rooting type, cottony type, dense mycelial type and concentric striate type. The mycelial growth rate was observed to be 15.8 – 428.8 mm^2/day on PDA medium and the decolorizing rate of monokaryons ranged from 11.23% to 89.54%. All monokaryotic isolates were used in determination of the mating type and it was noted that *P. pulmonarius* species has a bifactorial tetrapolar mating system. The result of interstrain crosses revealed that the numbers of A and B factors of three *P. pulmonarius* strains were 2 and 2, respectively. The mycelial growth rate and the decolorizing rate of most monokaryotic isolates was relatively high, thus, they could be used for breeding programs.

Keywords: Decolorizing rate, Mating types, Monokaryon, *P. pulmonarius*, PDA

Introduction

Phoenix mushroom (*Pleurotus pulmonarius*) is one of the most important cultivated mushrooms in Asian, Africa and Latin American countries

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(Zmitrovich and Wasser, 2016; Raman *et al*., 2021). At present, *P. pulmonarius* mushrooms is commonly grown in Vietnam especially at southern region. Phoenix mushroom is considered healthy because it contains high levels of protein, vitamins, and minerals (Silva *et al*., 2002; Okwulehie and Nosike, 2015). Mushroom species exhibited high values of biological efficiency and it is cultivated using a wide range of agro-substance, such as sawdust, cereals straw, sugarcane bagasse… with simple production techniques (Adebayo *et al*., 2014; Adewoyin and Ayandele, 2018; Kumla *et al*., 2020). Besides, phoenix mushroom can grow in a wide range of temperature, leading to high potential for commercial production of this mushroom species in tropical and subtropical regions (Chang and Miles, 2004; Zmitrovich and Wasser, 2016). Due to the above reasons, phoenix mushroom was selected to cultivate by most of mushroom growers in the Vietnam's southern provinces. Some common breeding objectives of oyster mushroom are higher yield, fruit body quality, sporelessness, resistance against abiotic and biotic stresses… (Chang and Miles, 2004; Barh *et al*., 2019). Several breeding techniques for strains improvement in *Pleutotus* including mutation breeding, genetic transformation, protoplast fusion, mycelial mating (Lee *et al*., 2011; Selvakumar *et al*., 2015; Barh *et al*., 2019). Among these, mating of monokaryotic mycelia is a common and simple technique to generate new strains (Lee *et al*., 2011).

P. pulmonarius is a bifactorial heterothallic mushroom species and the mating is controlled by two unlinked mating type factors (genes A and B) (Raper, 1978; Roach *et al*., 2014). Monokaryon is a fundamental breeding material in conventional procedures of mushroom crossbreeding. Monokaryon is a mycelium growing from a germinating sexual spore. Methods of obtaining monokaryons for *Pleurotus* have been described (Gharehaghaji *et al*., 2007; Abdulgani *et al*., 2017). However, screening monokaryons through evaluation of growth rate has not been discussed in the literature. Some screening methods including mycelial growth rate on media or determination of enzymatic activity could be applied. Besides, it is important to investigate the mating types of monokaryons for breeding programs. Clamp connection was used as a common indicator for identifying dikaryon formation of *Pleurotus*. Currently, the mating type of oyster mushrooms was usually determined by microscopy examination of clamp connection after mon - mon crossing.

In the present study, three *P. pulmonarius* strains originated from the southern of Vietnam were chosen to collect and determine monokaryotic isolates. Mycelial growth rate on agar medium and decolorizing rate of YBLB medium of these monokaryotic isolates were investigated. Mating types of monokaryons were determined by random hybridization.

Materials and methods

Fungal strains and condition for fruiting bodies

Three strains of phoenix mushroom (*P. pulmonarius*) were obtained from Institute of Applied Biotechnology, Ho Chi Minh City, Vietnam and maintained in malt yeast agar medium (MYA). These strains are commercial strains which were collected at 2 provinces in the southern Vietnam: ABI-F000241 strain from Vinh Long province, ABI-F000252 and ABI-F000253 strains from Dong Nai province.

Mycelia of these strains were cultured at 25℃ on sterile PDA medium (potato extract: 4 g/L, dextrose: 20 g/L, agar: 15 g/L) in the dark. Small pieces of vegetative cells (80 mm^2) obtained from the 7-day-old PDA were transferred to fruiting medium. This 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. After inoculation, the fully colonised bags were transferred into growth room of 85-95% humidity, 25-27℃ temperature. Fruiting bodies were collected just before sporulation.

Single basidiospore isolates and monokaryons

Single basidiospore isolation was done according to Gharehaghaji *et al.* (2007) with slight modifications. Single basidiospore isolates of each strain were collected from basidiocarps and diluted by sterile water to the concentration of 50-100 spores/mL (determined by hemocytometer). 100 µl of suspensions were spread on petri dishes with PDA medium and incubated at 25^oC. After incubation for 4-5 days, colony of each basidiospores were picked up by a needle and transferred into the fresh PDA medium. One week later, the mycelium was confirmed as monokaryon according to the absence of clamp connections under 40X magnifications of a microscope. 20 monokaryon isolates were obtained for each phoenix mushroom strain.

Study of the monokaryons growth rate on PDA medium

Small pieces of monokaryon isolates (5 mm^2) obtained from the 7-day-old PDA were cited in the centre of petri dishes (9 cm diameter) containing 20 mL of sterile PDA medium. The mycelium discs were incubated at 25^oC in the dark. The area of the hyphal expansion was measured after 10 days of incubation using the ImageJ program (National Institutes of Health, USA). The average growth rate was calculated using the formula given below:

$$\text{Growth rate } (mm^2/day) = \frac{\text{Mycelium area after 10 days of incubation (mm2)}}{\text{Incubation period (10 days)}}$$

Study of the monokaryons decolorizing rate on YBLB medium

Study of the decolorizing rate was done according to Magae *et al.* (2005) with slight modifications. Small pieces of monokaryon isolates (5 mm^2) obtained from the 7-day-old PDA were transferred to test tubes (16 mm diameter) containing 5 mL of sterile YBLB medium. This medium contained 4.5 g of yeast extract, 7.5 g of pepton, 5 g of lactose, 0.025 of bromothymol blue and 1000 mL of distilled water at a pH of 7.5. The cultures were incubated at 25^{o}C in static conditions for 4 days. The media were centrifuged at 4,000 × g for 2 min, and the OD values of the supernatants were determined at 615 nm. The decolorizing rate (expressed as a percentage) was calculated using the formula given below:

$$\text{Decolorizing rate (\%)} = \frac{\text{A615 of blank} - \text{A615 of strain}}{\text{A615 of blank}} \text{ x } 100$$

Where A615 of blank and A615 of strain are optical densities at 615 nm of blank and culture, respectively.

Determination of mating types of monokaryons

Mating types of monokaryon isolates from the same parent strain were determined by random crossing. Two mycelial agar disks (5 mm^2) were cut out from 10 days old monokaryotic isolates and placed 2 cm apart in the center of a petri dish containing PDA medium. They were incubated at 25^{o}C for about 1-2 weeks until two fronts of the advancing mycelia from the agar pieces met and formed a large contact zone. A small inoculum was taken from the contact zone of the paired monokaryon isolates and examined under a microscope (×40) for clamp connections. By analysis result of mating tests, the four expected groups (AxBx, AxBy, AyBx, AyBy) were determined for each phoenix mushroom strain. A monokaryon of each group was used as a tester representing for each phoenix mushroom strain in interstrain crosses.

Results

Monokaryotic isolates, mycelial growth rate on PDA medium and decolorizing rate on YBLB medium

The results showed that 60 monokaryotic isolates were collected (20 isolates each strain) and four types of monokaryon's colony morphology were observed: rooting type, cottony type, dense mycelial type and concentric striate type (Figure 1).

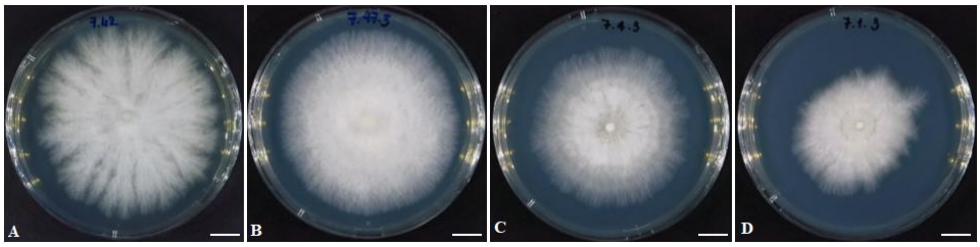

Figure 1. Morphological monokaryon types (*A: rooting type; B: cottony type; C: concentric striate type; D: dense mycelial type; bar: 1 cm*)

After 10 days of incubation, the results of mycelial growth rate on PDA medium and decolorizing rate on YBLB medium were presented in Table 1.

Table 1. Growth rates on PDA medium and decolorizing rate on YBLB medium of monokaryotic isolates

Strain ABI-F000241			**Strain ABI-F000252**			**Strain ABI-F000253**		
Isolate code	Growth rates (mm^2/day)	Decolorizing rate (%)	Isolate code	Growth rates (mm^2/day)	Decolorizing rate (%)	Isolate code	Growth rates (mm^2/day)	Decolorizing rate (%)
01	$70.5^{ij} \pm 1.35$	$58.83^{bcd} \pm 11.17$	**02**	$241.5^{d} \pm 34.1$	$67.54^{cde} \pm 14.41$	**01**	$165.7^{f} \pm 22.3$	$41.17^{gh} \pm 26.80$
04	$303.3^{cd} \pm 11.7$	$46.63^{efg} \pm 5.23$	**04**	$326.3^{b} \pm 34.2$	$20.96^{k} \pm 1.98$	**04**	$329.9^{b} \pm 10.5$	$49.78^{efgh} \pm 17.67$
05	$274.4^{d} \pm 13.1$	$29.02^{h} \pm 5.38$	**07**	$299.0^{c} \pm 19.4$	$50.42^{fgh} \pm 5.82$	**08**	$212.5^{ef} \pm 17.1$	$86.97^{a} \pm 9.28$
06	$66.8^{ij} \pm 6.2$	$58.43^{bcd} \pm 12.69$	**09**	$207.2^{e} \pm 24.3$	$80.48^{abc} \pm 3.45$	**09**	$171.3^{f} \pm 38.4$	$67.32^{abcdef} \pm 19.74$
08	$130.3^{g} \pm 14.0$	$56.59^{bcd} \pm 12.22$	**12**	$246.1^{d} \pm 24.8$	$83.46^{ab} \pm 2.39$	**13**	$238.6^{de} \pm 27.1$	$72.41^{abcde} \pm 17.06$
09	$58.4^{ij} \pm 3.7$	$52.55^{def} \pm 3.61$	**13**	$327.7^{b} \pm 7.4$	$19.72^{k} \pm 3.56$	**16**	$266.2^{cd} \pm 29.4$	$55.38^{defg} \pm 19.08$
13	$227.1^{e} \pm 29.5$	$34.43^{gh} \pm 16.04$	**15**	$399.2^{a} \pm 36.9$	$39.79^{h} \pm 26.96$	**20**	$323.3^{b} \pm 19.4$	$60.51^{bcdefg} \pm 27,26$
19	$206.8^{ef} \pm 26.9$	$67.76^{b} \pm 1.53$	**16**	$26.9^{k} \pm 8.7$	$79.58^{abc} \pm 4.52$	**23**	$390.5^{a} \pm 32.6$	$78.01^{abcd} \pm 18.75$

Strain ABI-F000241			Strain ABI-F000252			Strain ABI-F000253		
20	50.2^{jk} ± 4.2	39.49^{fgh} ± 7.31	**20**	163.4^{fg} ± 19.2	73.93$^{abc d}$ ± 13.23	**24**	205.0^{ef} ± 29.8	74.83$^{abc d}$ ± 15.48
23	65.1^{ij} ± 3.7	67.26^{b} ± 3.07	**22**	83.5^{h} ± 12.3	57.72^{efg} ± 5.22	**27**	341.7 ± 41.4	86.93^{a} ± 2.78
24	322,8^{bc} ± 55.2	40.35^{fgh} ± 21.06	**24**	41.7^{k} ± 9.9	87.02^{a} ± 6.33	**36**	401.8^{a} ± 24.3	81.02^{abc} ± 13.96
26	118.0^{gh} ± 41,0	85.29^{a} ± 1.85	**27**	179.1^{f} ± 13.4	71.31$^{bcd e}$ ± 6.10	**37**	164.7^{f} ± 58.2	48.49^{fgh} ± 14.48
33	81.9^{hij} ± 4.7	87.57^{a} ± 4,67	**29**	236.9^{d} ± 16.8	85.03^{ab} ± 4.05	**41**	335.4^{b} ± 66.4	59.22$^{cde fg}$ ± 19.92
34	191.8^{f} ± 37.2	88.16^{a} ± 2.31	**30**	27.0^{k} ± 2.0	76.02$^{abc d}$ ± 5.96	**42**	105.1^{f} ± 21.8	48.10^{fgh} ± 7.12
36	428.8^{a} ± 26.2	11.23^{i} ± 2.82	**31**	347.7^{b} ± 17.4	20.94^{k} ± 13.49	**44**	410.2^{a} ± 38.0	30.04^{hi} ± 12.44
37	20.9^{k} ± 4.0	52.75^{def} ± 15.60	**33**	350.6^{b} ± 14.8	63.04^{def} ± 5.55	**45**	390.9^{a} ± 24.3	14.78^{i} ± 8.85
43	95.8^{ghi} ± 6.0	51.53^{def} ± 10.32	**34**	181.1^{f} ± 11.1	37.17^{h} ± 13.16	**47**	340.0^{b} ± 57.3	14.29^{i} ± 4.66
45	110.9^{gh} ± 5.8	89.54^{a} ± 2.72	**36**	141.1^{g} ± 15.7	37.17^{h} ± 12.46	**51**	309.4^{bc} ± 28.0	73.58$^{abc de}$ ± 2.57
59	353.1^{b} ± 66.9	58.78^{bcd} ± 19.44	**39**	327.7^{b} ± 21.5	48.82^{gh} ± 10.26	**52**	246.0^{de} ± 31.2	70,26$^{abc def}$ ± 3.35
60	15.8^{k} ± 2.1	61,92^{bc} ± 22.81	**43**	37,3^{k} ± 3,8	84.42^{ab} ± 4.62	**54**	75,2^{f} ± 13.8	83,46^{ab} ± 2.69

* 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.

In this study, the effects of monokaryotic isolates on mycelial growth rate and decolorizing rate were investigated. The results showed that the mycelial growth rate of 60 monokaryotic isolates were different and specific for each mushroom strain. The mycelial growth rate of ABI-F000241 and ABI-F000252 monokaryons ranged from 15.8 mm^2/day to 428.8 mm^2/day and from 26.9 mm^2/day to 399.2 mm^2/day, respectively**,** while growth rate of ABI-F000253 was in the range of 75.2 mm^2/day to 410.2 mm^2/day (Table 1 and Figure 2, 3, 4).

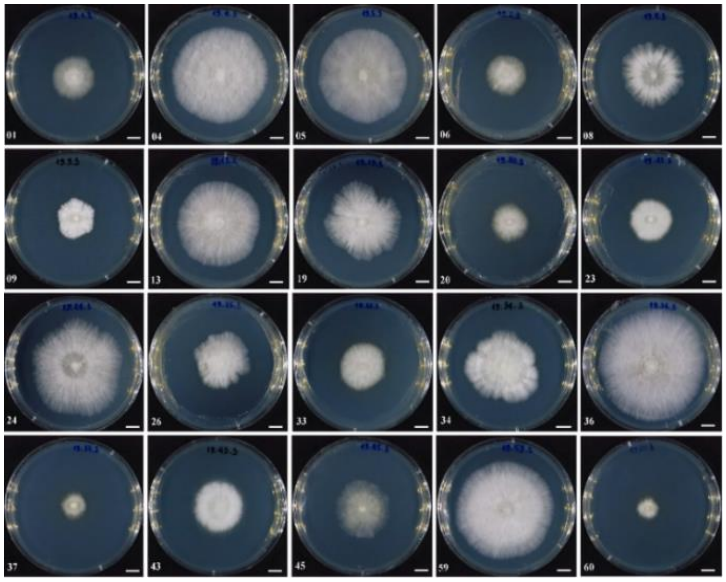

Figure 2. Colony morphology of monokaryotic isolates (ABI-F000241 strain) on PDA medium after 10 days of incubation (bar: 1 cm)

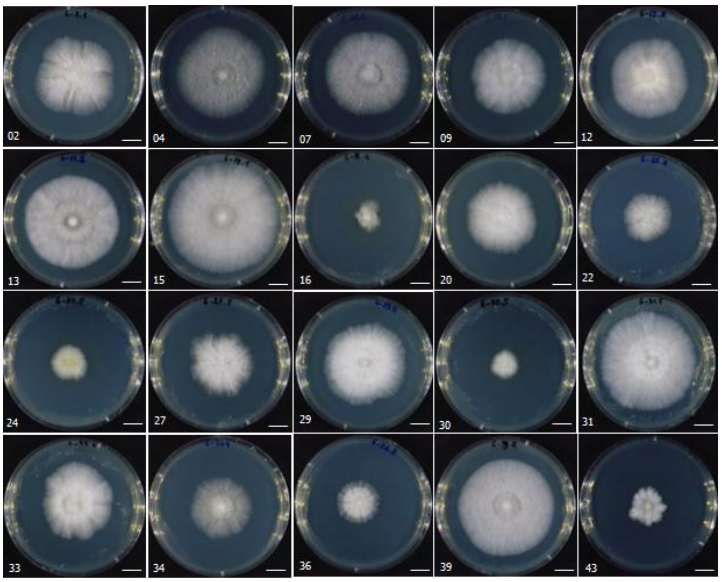

Figure 3. Colony morphology of monokaryotic isolates (ABI-F000252 strain) on PDA medium after 10 days of incubation (bar: 1 cm)

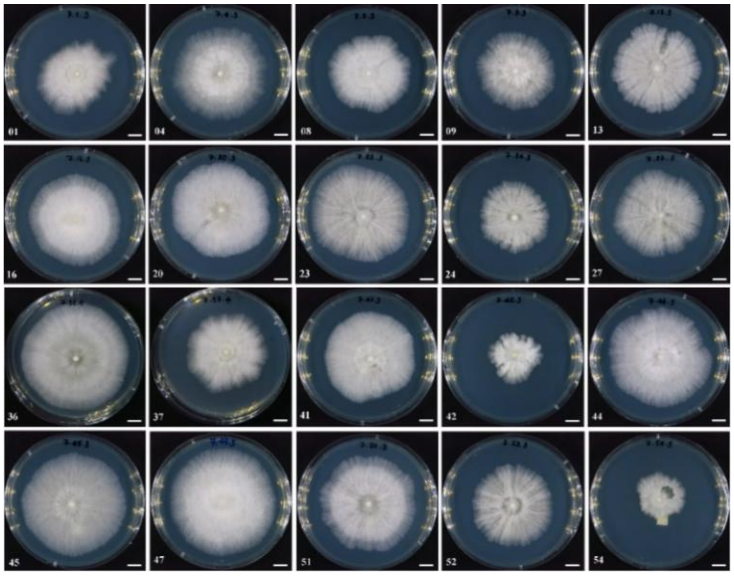

Figure 4. Colony morphology of monokaryotic isolates (ABI-F000253 strain) on PDA medium after 10 days of incubation (bar: 1 cm)

The decolorizing rate on YBLB medium of monokaryotic isolates was significantly different and specific for each mushroom strain. The colour of media changed from green to yellow (Figure 5, 6, 7).

The decolorizing rate of ABI-F000241 and ABI-F000252 monokaryons ranged from 11.23% to 89.54% and from 19.72% to 87.02%, respectively**,** while decolorizing rate of ABI-F000253 was in the range of 14.29 % to 86.97 % (Table 1).

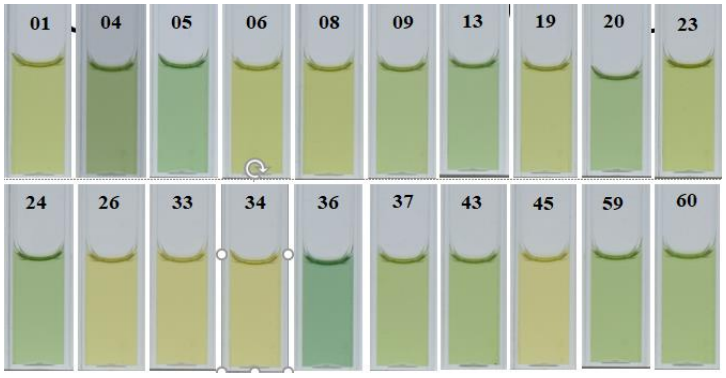

Figure 5. Characterization of YBLB medium inoculated with *P. pulmonarius* monokaryotic isolates (ABI-F000241 strain)

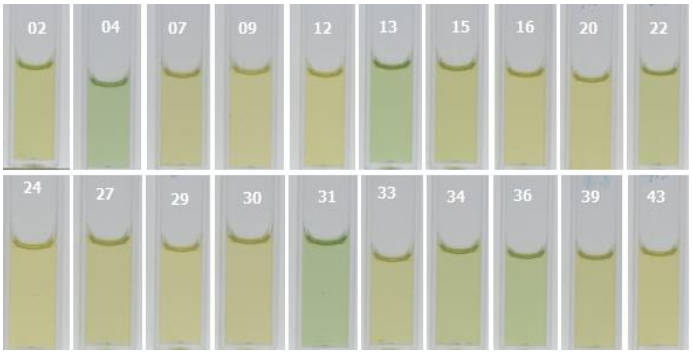

Figure 6. Characterization of YBLB medium inoculated with *P. pulmonarius* monokaryotic isolates (ABI-F000252 strain)

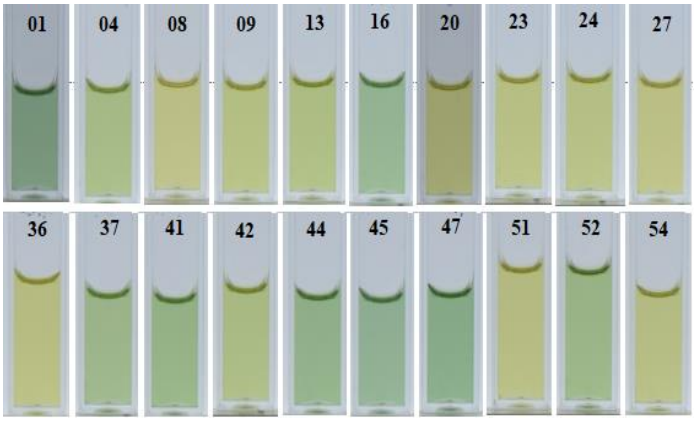

Figure 7. Characterization of YBLB medium inoculated with *P. pulmonarius* monokaryotic isolates (ABI-F000253 strain)

Mating types of monokaryons

The results of mating test were presented in Tables 2, 3, 4. ABI-F000241 strain had 3 monokaryotic isolates type A1B1, 7 monokaryotic isolates type A2B2, 5 monokaryotic isolates type A1B2 and 5 monokaryotic isolates type A2B1. ABI-F000252 strain had 8 monokaryotic isolates type A1B1, 5 monokaryotic isolates type A2B2, 2 monokaryotic isolate type A1B2 and 5 monokaryotic isolate type A2B1. ABI-F000253 strain had 5 monokaryotic isolates type A1B1, 4 monokaryotic isolates types A2B2, 5 monokaryotic isolates type A1B2 and 6 monokaryotic isolates type A2B1.

Table 2. Intrastrain matings result between monokaryons of ABI-F000241

		A_1B_1	A_1B_2	A_2B_1	A_2B_2
		01 05 08	04 20 26 37 60	13 19 23 36 45	06 09 24 33 34 43 59
A_1B_1	01 05	- - -	- - -	(+) (+) (+)	+ + +
	08	- - -	- - -	(+) (+) (+)	+ + +
A_1B_2	04 20	- - -	- - -	+ + +	(+)(+)(+)
	26 37 60	- - -	- - -	+ + +	(+)(+)(+)
		- - -	- - -	+ + +	(+)(+)(+)
A_2B_1	13 19	(+)(+)(+)	+ + +	- - -	- - -
	23 36 45	(+)(+)(+)	+ + +	- - -	- - -
		(+)(+)(+)	+ + +	- - -	- - -
A_2B_2	06 09	+ + +	(+) (+) (+)	- - -	- - -
	24 33	+ + +	(+) (+) (+)	- - -	- - -
	43 59	+ + +	(+) (+) (+)	- - -	- - -

Note: +: clamp connections formed; (+): pseudo- clamp connections formed; -: no clamp connections formed

Table 3. Intrastrain matings result between monokaryons of ABI-F000252 strain

		A_1B_1	A_1B_2	A_2B_1	A_2B_2
		02 04 12 13 15 20 22 24	27 29	07 09 31 39 43	16 30 34 33 36
A_1B_1	02 04 12	- - -	- -	(+) (+) (+)	+ + +
	13 15 20	- - -	- -	(+) (+) (+)	+ + +
	22 24	- - -	- -	(+) (+) (+)	+ + +
A_1B_2	27 29	- - -	- -	+ + +	(+)(+)(+)
A_2B_1	07 09 31	(+) (+) (+)	+ +	- - -	- - -
	39 43	(+) (+) (+)	+ +	- - -	- - -
A_2B_2	16 30 33	+ + +	(+) (+)	- - -	- - -
	34 36	+ + +	(+) (+)	- - -	- - -

Note: +: clamp connections formed; (+): pseudo- clamp connections formed; -: no clamp connections formed

Table 4. Intrastrain matings result between monokaryons of ABI-F000253 strain

		A_1B_1	A_1B_2	A_2B_1	A_2B_2
		04 08 09 36 54	01 20 23 24 37	16 41 42 44 47 52	13 27 45 51
A_1B_1	04 08	- - -	- - -	(+) (+) (+)	+ + +
	09 36	- - -	- - -	(+) (+) (+)	+ + +
	54	- - -	- - -	(+) (+) (+)	+ + +
A_1B_2	01 20	- - -	- - -	+ + +	(+) (+) (+)
	23 24	- - -	- - -	+ + +	(+) (+) (+)
	37	- - -	- - -	+ + +	(+) (+) (+)
A_2B_1	16 41	(+) (+) (+)	+ + +	- - -	- - -
	42 44	(+) (+) (+)	+ + +	- - -	- - -
	47 52	(+) (+) (+)	+ + +	- - -	- - -
A_2B_2	13 27	+ + +	(+)(+)(+)	- - -	- - -
	45 51	+ + +	(+)(+)(+)	- - -	- - -

Note: +: clamp connections formed; (+): pseudo- clamp connections formed; -: no clamp connections formed

To determinate mating relationship between three *P. pulmonarius* strains, interstrain crosses were made with respective monokaryotic tester isolates. For ABI-F000241 strain, tester isolates were selected as follows: A1B1 = isolate 241.08; A1B2 = isolate 241.26; A2B1 = isolate 241.13; A2B2 = isolate 241.24. For ABI-F000252 strain, tester isolates were selected as follows: A1B1 = isolate 252.02; A1B2 = isolate 252.29; A2B1 = isolate 252.31; A2B2 = isolate 252.16. For ABI-F000253 strain, tester isolates were selected as follows: A1B1 = isolate 253.08; A1B2 = isolate 253.23; A2B1 = isolate 253.16; A2B2 = isolate 253.27. In interstrain crosses, each monokaryotic tester isolate of each strain was out-crossed to monokaryotic tester isolates of others strains. The results of interstrain crosses were presented in Table 5.

As a result of these crosses, the numbers of A and B factors of three *P. pulmonarius* strains were 2 and 2, respectively. Mating types of all strains were A1B1, A1B2, A2B1, A2B2.

Table 5. Interstrain matings result of tester strains between three *P. pulmonarius* strains

Isolate code	**241. 0**8	241. 26	241. 13	241. 24	252. 02	252. 29	252. 31	252. 16
241.08					-	-	-	+
241.26					-	-	+	-
241.13					-	+	-	-
241.24					+	-	-	-
252.02								
252.29								
252.31								
252.16								
253.08	-	-	+	-	-	-	+	-
253.23	-	-	-	+	-	-	-	+
253.16	+	-	-	-	+	-	-	-
253.27	-	+	-	-	-	+	-	-

Note: +: clamp connections formed; -: no clamp connections formed

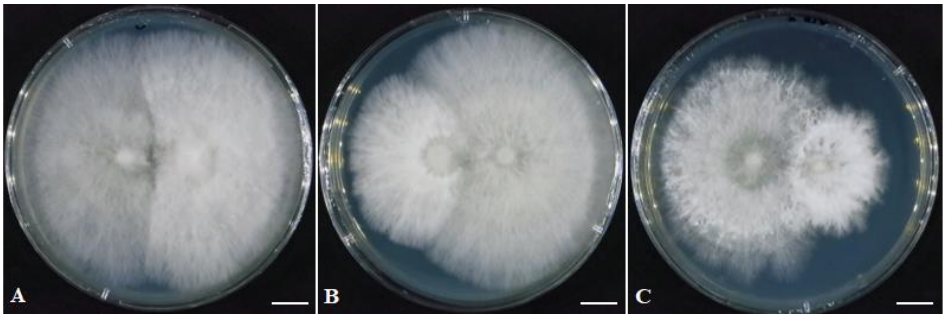

Figure 8. Three types of touching breeding of *P. pulmonarius (A)- borderline type, (B)- surrounding type, (C)- edge type*

Discussion

Colony morphology *of* monokaryons in this study was similar to previous reports. My (2018) reported that there were 4 monokaryon's colony types: rooting, cotton, dense mycelial and concentric striate of three *Pleutotus* species: *P. ostreatus, P. pulmonarius, P. citrinopileatus.* In another report by Lee *et al.* (2012), colony morphology of *P. ostreatus* monokaryons was observed to be fluffy, puffy, concentric, feathery, streak to cumulous.

PDA is a popular medium for growth of both dikaryotic and monokaryotic mycelia. In this study, the mycelial growth rates of different isolates were different for each strain. The growth rate of monokaryotic isolates in the present study were 15.8 – 428.8 (mm^2/day), which was similar to previous reports. Patel *et al*., (2015) reported that monokaryotic mycelial growth rate of *P. ostreatus* was from 1.1 to 5.0 mm/day after 7 days of incubation (6.65 to 137.38 mm^2/day), while Anderson *et al.* (1973) showed that it was 3.4 mm/day (15.88 mm^2/day). Monokaryotic mycelium grew slower compared to those of original parental dikaryon, which was in agreement with findings of Anderson *et al.* (1973), Gaitán-Hernández and Salmones (2008), Lee *et al.* (2012) and My. (2018).

Based on colour changes in the YBLB medium, Magae *et al*. (2005) determined the degeneration of enoki mushroom (*F. velutipes*). They observed a colour change to yellow in culture media of good quality strains. In contrast, the degenerated strains have low decolorizing rate in the YBLB media so the colour of media did not change (stayed in blue). A similar finding was reported by Chen *et al*. (2019), who identified that the decolorizing rate of paddy straw mushroom (*V. volvacea*) strains in YBLB media weakened as it degenerated. In our study, the decolorizing rate of most monokaryotic isolates was relatively high. Therefore, they could be used for breeding programs.

Three types of touching breeding at the contact zone were observed: edge type, surrounding type and borderline type (Figure 8). In edge type, mycelia produced aerial hyphae that resulted in a puffy appearance of the colony and clamp connections were observed (compatible matings). However, breeding pairs made surrounding and borderline type were not compatible (no clamp connections formed), which was also reported by Gharehaghaji *et al.* (2007), Lee *et al.* (2012) and My (2018).

Using mating experiments, the compatible groups of all monokaryotic isolates were determined. It was noted that *P. pulmonarius* species has a bifactorial tetrapolar mating system as other *Pleurotus* species with a large number of factors at both the A and B loci. Eugenio and Anderson (1968) reported that 60 A and 190 B alleles was estimated for *P. ostreatus*. In another report by Anderson *et al*. (1991), *P. ostreatus* had 126 A alleles and 354 B alleles. In this study, because the numbers of A and B factors of the three strains were 2 and 2, these strains share the same origin.

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