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https://doi.org/10.5109/2558889

出版情報：九州大学大学院農学研究院紀要. 65 (1), pp.35-44, 2020-02. Faculty of Agriculture, Kyushu University
バージョン：
権利関係：

J. Fac. Agr., Kyushu Univ., **65** (1), 35–44 (2020)

Biological Characteristics and Cultivation of the Wild Edible Mushroom *Pleurotus dryinus*

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(*Received October 28, 2019 and accepted November 14, 2019*)

The biological characteristics and domestication cultivation of *Pleurotus dryinus* were studied by single factor test and orthogonal test using the wild strains of *P. dryinus* from Horqin Left-wing Back Banner of Tongliao City, Inner Mongolia Autonomous Region, China. According to these experiments, the optimal medium for mycelial growth was obtained. Namely, D (+)–maltose, $NaNO_3$, K_2HPO_4 and straw. Based on the single factor experiment of temperature and pH, 22˚C and pH9 were suggested that good growth of mushrooms.

In the domestication experiments of *P. dryinus*, the phases of pre-culture spawn, of manufacturing cultural bags, of spawn running, of inducement to primordium, of fruiting period management, of collected periods were studied.

Key words: domestication, orthogonal test, *Pleurotus dryinus*, single factor

INTRODUCTION

Numerous species of the genus *Pleurotus* (Fr.) P. Kumm. are very important edible and medical mushrooms (Wu *et al*., 2019). Several of them are cultivated in large commercial scales (Sánchez, 2010). Due to their importance, plenty of researches has been carried out on this genus (Corner, 1981; Pegler, 1996; Bau *et al*., 2001; Albertó *et al*., 2002; Karunarathna *et al*., 2012; Li *et al*., 2014; Liu *et al*., 2015; Li *et al*., 2017).

Pleurotus dryinus (Pers.) P. Kumm. is a delicious edible mushroom when young, meanwhile it has a strong fragrance after dried (Zhao *et al*., 2001). Also it has certain medicinal value can be used to treat emphysema (Dai *et al*., 2009). Because of its great taste and rich nutrition, Coupled with its potential medicial value, *P. dryinus* is popular in current China edible mushroom market. Until now, most studies on *P. dryinus* have focused on enzyme production (Ivana *et al*., 2006; Elisashvili *et al*., 2006, 2008; Yoon *et al*., 2014; Maftoun *et al*., 2015). There is a lack of researches on domestication culture, formula selection and heredity genetic breeding (Huang *et al*., 2001; Yao *et al*., 2003).

Using the materials collected from tongliao city, Inner Mongolia autonomous region, this paper carried out the biological characteristics and cultivation, meantime provided basic data for the conservation and development of this precious fungal resource.

MATERIALS AND METHODS

Fungal strain

Specimens were collected from Sandy land poplar roots in Horqin Left–wing Back Banner of Tongliao City, Inner Mongolia Autonomous Region, China. The strain was originally isolated from the fresh fruit body, was numbered MCCJLAU0380 and deposited in strains library of fungi diversity laboratory which are attached to engineering research center of Chinese ministry of education for edible and medicinal fungi in Jilin agricultural university. This strain with obvious chlamydospores is easily distinguished from other strains, and molecular data supports this conclusion.

The basal medium and culture conditions

By aseptic processing, the strains were transferred to Petri dishes which containing 20 ml medium (potato 200 g, glucose 20 g, agar 20 g, KH_2PO_4 1 g, $MgSO_4$ 0.5 g, vitamin B_1 100 mg, ddH_2O 1000 ml). Then these Petri dishes are placed at 25˚C in darkness. When the mycelial grows to half of the Petri dishes, it was punched into 8 mm homogeneous pieces at the periphery of colonies by the puncher. These pieces of mycelia were used for the next experiments.

The basal medium A (g/L) : carbon source 20 g, yeast powder leaching 2 g, KH_2PO_4 1 g, $MgSO_4$ 0.5 g, agar 20 g, The solution is distilled water. The basal medium B (g/L) : glucose 20 g, nitrogen source 2 g, KH_2PO_4 1 g, $MgSO_4$ 0.5 g, agar 20 g, The solution is distilled water. The basal medium C (g/L) : glucose 20 g, yeast powder leaching 2 g, $MgSO_4$ 0.5 g, agar 20 g, The solution is distilled water. The basal medium D (g/L) : glucose 20 g, yeast powder leaching 2 g, vitamin B_1 100 mg, agar 20 g, The solution is distilled water. The basal medium E (g/L) : carbon source 20 g, nitrogen source 2 g, inorganic salt 1 g, growth factor 30 g/L, agar 20 g, The solution is distilled water. The basal medium F (g/L) : glucose

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20 g, yeast powder leaching 2 g, KH_2PO_4 1 g, $MgSO_4$ 0.5 g, vitamin B_1 100 mg, agar 20 g, The solution is distilled water.

These medium was autoclaved at 121˚C for 30 min. The mycelia were inoculated into the solidified medium under test by aseptic operation. Then the culture dishes were incubated at 25˚C in the dark. All experiments were performed in sextuple. Mycelial diameter and mycelial growth are observed, measured and recorded every 48h after 72 h inoculation.

Single factor test of carbon and nitrogen source

In the test of carbon source, 2% addition level of glucose, D–fructose, soluble starch, sucrose, soybean meal, malt powder leaching, D (+)–maltose, glycine, α–lactose, D–mannitol, lactose and D–mannose was added individually to the basal medium A. The medium without carbon source was used as the control medium.

In the test of nitrogen source, 0.2% addition level of LB–broth, $(NH_4)_2HPO_4$, beef powder, peptone, urea, yeast powder leaching, yeast extract, $NaNO_3$, KNO_3, $(NH_4)_2SO_4$, NH_4NO_3 and beef paste was added individually to the basal medium B. The medium without nitrogen source was used as the control medium.

Single factor test of inorganic salt and growth factor

In the test of inorganic salt, both macroelement and microelement were tested. 0.1% addition level of K_2HPO_4, KH_2PO_4, Ca $(H_2PO_4)_2$, $CaCl_2$, NaCl, $MgSO_4$, KCl, $MgCl_2$, Na_2HPO_4 and NaH_2PO_4 was added individually to the basal medium C. 0.001% addition level of $Zn_3(PO_4)_2$, $FeCl_3$ and $ZnCl_2$ was added individually to the basal medium C. The medium C was used as the control medium.

In the test of growth factor, vitamin and natural substances were tested. 0.01% addition level of VB_1, VB_2 and VC was added individually to the basal medium D to replace the VB_1. Eight natural components (potato, bean sprouts, wheat, straw, *Pleurotus ostreatus*, corn, oak wood and carrots) which was severally weighed 30 g/L, boiled the juice and filtered was added individually to the basal medium D to replace the VB_1. The medium without growth factor was used as the control medium (Lu *et al*., 2017).

Effect of orthogonal text on mycelial growth

Orthogonal test was used to obtain the optimum medium for mycelial growth. Through the single factor test of carbon source, nitrogen source, inorganic salt and growth factor, three best growth levels of the four factors were obtained. Based on these data, the orthogonal experiment is designed and operated. The basal medium E was selected as the basic medium.

Single factor test of temperature

In the test of temperature, five temperatures were selected to determine the most suitable temperature of mycelial growth. The basal medium F was used as the basal medium. These Petri dishes were put to incubator at 17˚C, 22˚C, 25˚C, 28˚C and 33˚C after inoculation, individually.

Single factor test of pH

In the test of pH, seven pH were selected to determine the most suitable pH of mycelial growth. The basal medium F was used as the basal medium. These mediums were adjusted to 4, 5, 6, 7, 8, 9 and 10 by using 1 mol/l CH_3COOH and 1 mol/l NaOH.

Single factor test for moisture content

In the test of moisture content, seven moisture contents were selected to determine the most suitable moisture content of mycelial growth. 5 g sawdust as a test basis, by increasing the amount of distilled water, the moisture content of sawdust respectively reached 45%, 50%, 55%, 60%, 65% and 70%.

Domesticated cultivation experiment

In the domestication experiments of *P. dryinus*, the phases of pre–culture spawn, of manufacturing cultural bags, of spawn running, of inducement to primordium, of fruiting period management and of collected periods were studied.

Analysis of data

All the data obtained from this experiment were analysed by analysis of variance and the tests of significance were determined by Duncan's multiple range tests.

RESULT AND DISCUSSIONS

Effect of carbon sources and nitrogen sources on mycelia growth

Carbon source has a significant effect on the mycelial growth of *Pleurotus dryinus*. The wild mushroom was isolated from fresh fruit body can grow on 13 kinds of culture medium. From the perspective of biochemistry, *P. dryinus* can use a variety of carbon sources to meet nutritional needs. Carbon sources are carbon nutrients used to form cells and participate in carbon–related reactions in metabolism. Their main functions are to form cell substances and provide energy of growth and development. According to the test results (Table 1, Fig. 1), D (+)–maltose showed the highest growth rate, followed by soybean meal and soluble starch. These groups were significantly higher than other control group. Above comprehensive consideration of mycelial growth rate and mycelial growth vigor, D (+)–maltose, soybean meal and soluble starch were selected as three levels of carbon sources in the orthogonal test.

The strain can be grown on 12 mediums. From the perspective of biochemistry, *P. dryinus* can use organic nitrogen and inorganic nitrogen to meet the nutritional needs. According to the test results (Table 2, Fig. 2), $NaNO_3$ had the highest growth rate, followed by $(NH_4)_2HPO_4$, lb–broth, KNO_3, peptone. Based on the comprehensive consideration of mycelial growth rate and mycelial growth vigor, $NaNO_3$, $(NH_4)_2HPO_4$ and LB–

Table 1. Effect of carbon sources on mycelial growth of *Pleurotus dryinus*

	Carbon source	Mycelial growth rate (mm/d)*	Significance levels		Mycelial growth vigor (mm/d)**
			0.05	0.01	
1	glucose	1.02±0.10	f	E	+
2	D–fructose	1.29±0.40	f	DE	+
3	soluble starch	5.95±1.09	b	B	+++
4	sucrose	3.33±0.51	d	C	++
5	D(+)–maltose	8.42±0.44	a	A	++
6	soybean meal	5.69±0.54	b	B	+++
7	malt powder leaching	4.65±0.57	c	B	+++
8	control	5.12±0.80	bc	B	+++
9	glycine	2.45±0.81	de	CD	++
10	α–lactose	1.42±0.40	f	DE	+
11	D–mannitol	3.19±0.43	d	C	+
12	lactose	1.24±0.22	f	DE	+
13	D–mannose	1.79±0.15	ef	DE	+

* Date represent the average values of six replicates.
** "+++" vigorous mycelial growth, "++" intermediate mycelial growth, "+" weak mycelial growth.

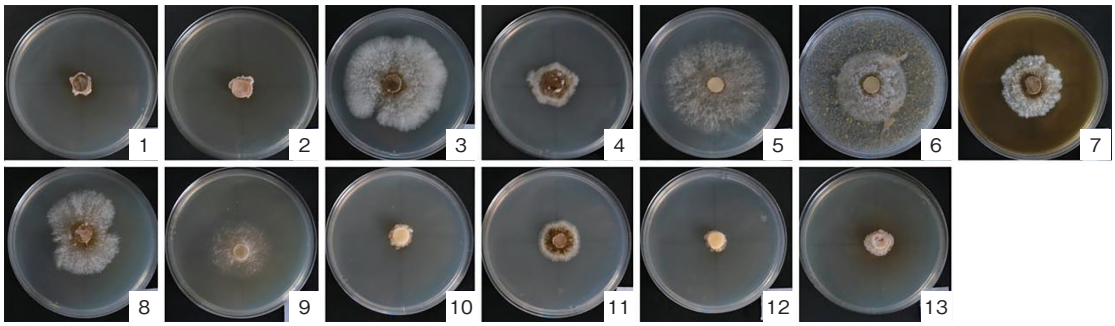

Fig. 1. Effect of carbon sources on mycelial growth of *Pleurotus dryinus*. (Photos on the 15th day.)

Table 2. Effect of nitrogen sources on mycelial growth of *Pleurotus dryinus*

	nitrogen source	Mycelial growth rate (mm/d)*	Significance levels		Mycelial growth vigor (mm/d)**
			0.05	0.01	
1	control	2.01±0.28	d	C	+
2	$(NH_4)_2HPO_4$	4.91±0.70	a	A	+++
3	LB – broth	4.38±0.59	b	A	+
4	beef powder	1.74±0.33	de	CD	++
5	peptone	2.05±0.28	d	C	++
6	urea	0	g	F	
7	yeast powder leaching	1.35±0.10	ef	D	+
8	yeast extract	1.52±0.32	ef	CD	++
9	$NaNO_3$	4.99±0.70	a	A	+
10	KNO_3	2.95±0.25	c	B	++
11	$(NH_4)_2SO_4$	0.65±0.24	g	E	+
12	NH_4NO_3	1.34±0.10	ef	D	+
13	beef paste	1.21±0.01	f	DE	+

*, ** As in Table 1

broth were selected as three carbon sources of the orthogonal test.

Effect of inorganic salt and growth factor on mycelial growth

Mycelial can grow on 13 culture mediums (Table 3, Fig. 3). The main functions of macroelement are to participate in the composition of cellular substances and enzymes, maintain the role of enzymes, control the gelation of protoplasm and regulate cell osmotic pressure. The function of microelement is to act as the components or activators of enzyme active groups. K_2HPO_4, Na_2HPO_4, NaCl and KCl promotes mycelial growth, KH_2PO_4, $Ca(H_2PO_4)_2$, $CaCl_2$, $MgSO_4$, $MgCl_2$, NaH_2PO_4, $FeCl_3$ and $ZnCl_2$ inhibits mycelial growth, mycelial didn't grow on the medium with $Zn_3(PO_4)_2$. After comprehensive consideration of mycelial growth rate and mycelial growth vigor, K_2HPO_4, Na_2HPO_4 and NaCl were selected as three levels of inorganic salts in the orthogonal test.

There are obvious effects on the mycelial growth of *P. dryinus*. The strain can grow on 12 kinds of culture mediums. Based the test results (Table 4, Fig. 4), straw, *P. ostreatus*, corn, potato, bean sprouts and carrots are very good growth factors and have good advantage over

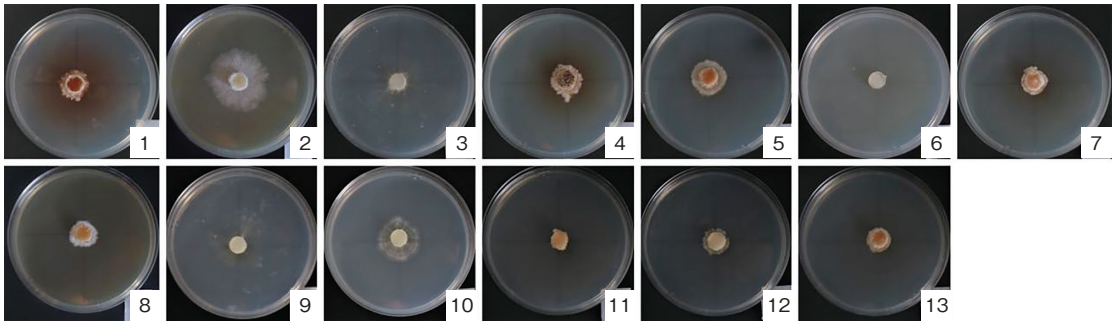

Fig. 2. Effect of nitrogen sources on mycelial growth of *Pleurotus dryinus*. (Photos on the 15th day.)

Table 3. Effect of inorganic salt on mycelial growth of *Pleurotus dryinus*

	inorganic salt	Mycelial growth rate (mm/d)*	Significance levels 0.05	0.01	Mycelial growth vigor (mm/d)**
1	K_2HPO_4	3.37±0.60	a	A	+++
2	KH_2PO_4	1.02±0.14	de	CD	+
3	$Ca(H_2PO_4)_2$	1.16±0.11	cde	CD	+
4	$CaCl_2$	1.21±0.13	cde	CD	+
5	NaCl	1.45±0.18	c	C	+
6	$MgSO_4$	1.22±0.12	cde	CD	+
7	KCl	1.36±0.32	cd	CD	+
8	$MgCl_2$	1.23±0.08	cde	CD	+
9	Na_2HPO_4	1.96±0.61	b	B	+
10	NaH_2PO_4	0.88±0.31	e	D	+
11	$Zn_3(PO_4)_2$	0	f	E	+
12	$FeCl_3$	1.11±0.15	cde	CD	+
13	$ZnCl_2$	1.10±0.22	cde	CD	+
14	control	1.24±0.06	cde	CD	+

*, ** As in Table 1

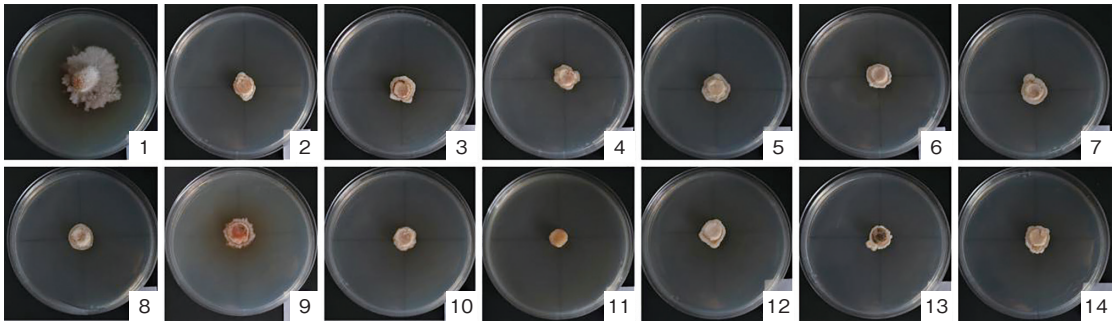

Fig. 3. Effect of inorganic salt on mycelial growth of *Pleurotus dryinus*. (Photos on the 15th day.)

Table 4. Effect of growth factor on mycelial growth of *Pleurotus dryinus*

	growth factor	Mycelial growth rate (mm/d)*	Significance levels 0.05	0.01	Mycelial growth vigor (mm/d)**
1	VB_1	1.74±0.31	g	FG	++
2	VB_2	1.38±0.46	g	G	+
3	VC	1.26±0.35	g	G	+
4	potato	4.14±0.50	d	D	+++
5	bean sprouts	2.93±1.02	e	E	+++
6	wheat	1.86±0.33	fg	EFG	+
7	straw	13.67±0.10	a	A	+++
8	*Pleurotus ostreatus*	8.86±0.86	b	B	+++
9	corn	5.75±0.44	c	C	+++
10	oak wood	1.20±0.22	g	G	+
11	carrots	2.64±0.51	ef	EF	++
12	control	1.71±0.16	g	FG	+

*, ** As in Table 1

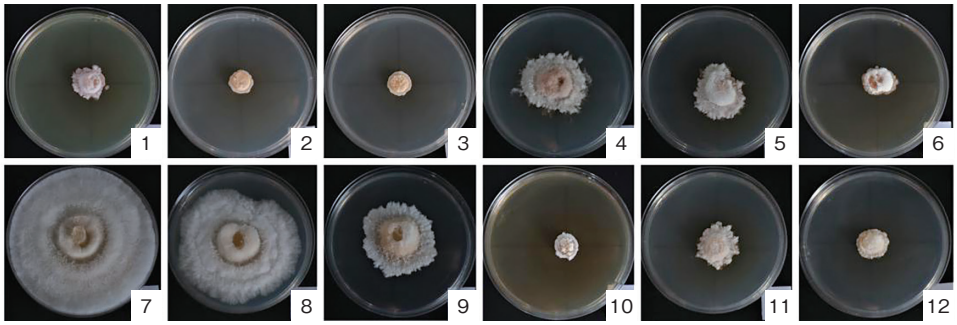

Fig. 4. Effect of growth factor on mycelial growth of *Pleurotus dryinus*. (Photos on the 15th day.)

other growth factor. After comprehensive consideration of mycelial growth rate and mycelial growth vigor, straw, *P. ostreatus* and corn were selected as three levels of growth factor in the orthogonal test.

The result of the orthogonal experiment

The purpose of the orthogonal test was to obtain the optimum medium (Table 5, Table 6, Fig. 5). The maximum difference of growth factors is 3.61, indicating growth factors is an important factor of mycelial growth, followed by nitrogen source, carbon source and inorganic salt. The first test not only had a significant growth rate than any other control, but also had better mycelial growth vigor than any test.

The F value of growth factor was the highest, followed by nitrogen source, carbon source and inorganic salt. Therefore, the significance difference of the four factors is growth factor, nitrogen source, carbon source, inorganic salt, successively. This is consistent with the results of intuitive analysis.

Effect of temperature and pH on mycelial growth

According to the single factor test result of temperature (Table 7, Fig. 6), the strain can grow at 17°C–33°C. When the temperature was 22°C, the mycelial growth rate and growth vigor were the best. Besides 17°C and 33°C have obvious distinctness and slower growth rate compared with 22–28°C. According to the single factor test result of pH (Table 8, Fig. 7), the growth of mycelial under alkaline condition is denser and whiter than that under acidic condition. When pH was 9, the mycelial grow rate and mycelial grow vigor were the best.

Single factor test for moisture content

According to the data (Table 9), it can be easily known that mycelial grow was the fastest when the mycelial was 55%. With the increase of water content, the growth rate of mycelial gradually slows down.

Cultivation

Pre–culture spawn

Solid medium: corn boiled with distilled water until didn't have no white heart, then filled bottle.

Liquid medium (g/L) : yeast powder leaching 10 g, glucose 10 g, sucrose 20 g, KH_2PO_4 1 g, $MgSO_4$ 0.5 g, The solution is distilled water.

These mediums were autochclaved at 121°C for 30 min. After inoculation, the solid spawn was cultured at 25°C in darkness until bottle was fulled with mycelial; the liquid spawn was cultured at 25°C and 150 r/min in darkness for 7 days (Zhang *et al*., 2018) (Fig. 8).

Table 5. Orthogonal test results of carbon source, nitrogen source, inorganic salt and growth factor

Test Number	Carbon source	Nitrogen source	Inorganic salt	Growth factor	Mycelial growth rate (mm/d)*	Mycelial growth vigor (mm/d)**
1	D (+)–maltose	$NaNO_3$	K_2HPO_4	straw	16.28±0.21	+++
2	D (+)–maltose	$(NH_4)_2{\cdot}HPO_4$	Na_2HPO_4	*Pleurotus ostreatus*	7.92±0.73	+++
3	D (+)–maltose	LB – broth	NaCl	corn	10.25±0.86	++
4	soybean meal	$NaNO_3$	Na_2HPO_4	corn	10.68±1.05	+++
5	soybean meal	$(NH_4)_2{\cdot}HPO_4$	NaCl	straw	8.31±0.73	+++
6	soybean meal	LB – broth	K_2HPO_4	*Pleurotus ostreatus*	8.21±0.64	+++
7	soluble starch	$NaNO_3$	NaCl	*Pleurotus ostreatus*	8.19±0.44	+++
8	soluble starch	$(NH_4)_2{\cdot}HPO_4$	K_2HPO_4	corn	8.68±0.77	+++
9	soluble starch	LB – broth	Na_2HPO_4	straw	10.58±0.51	+++
T1	206.68	210.88	199.03	210.96		
T2	163.17	149.47	175.06	145.98	T=89.10	
T3	164.73	174.21	160.47	177.62		
X1	11.48	11.72	11.06	11.72		
X2	9.07	8.30	9.73	8.11		
X3	9.15	9.68	8.92	9.87		
R	2.42	3.41	2.14	3.61		

Table 6. The results of F–test mycelial growth

Source	Type III Sum of Squares	df	MS	F value	Significance
Carbon source	56.41	2	28.20	24.96	0.0001
Nitrogen source	88.40	2	44.20	39.11	0.0001
Inorganic salt	35.09	2	17.55	15.53	0.0001
Growth factor	97.77	2	48.89	43.26	0.0001
Error	40.69	36	1.13		
Total	318.36	44			

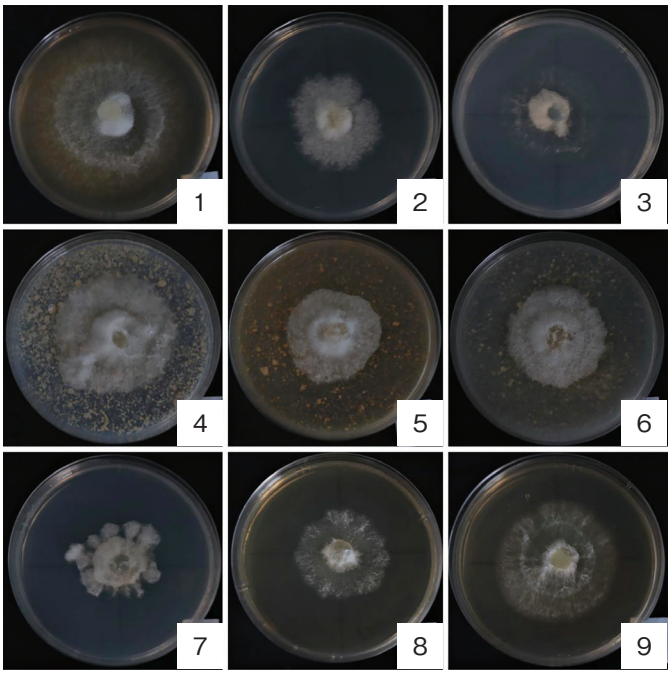

Fig. 5. Orthogonal test. (Photos of mycelium culture on the 11th day.)

Table 7. Effect of temperature on mycelial growth of *Pleurotus dryinus*

Temperature		Mycelial growth rate (mm/d)*	Significance levels 0.05	0.01	Mycelial growth vigor (mm/d)**
1	17℃	1.33±0.30	c	C	+
2	22℃	2.56±0.96	a	A	++
3	25℃	2.07±0.30	ab	ABC	++
4	28℃	2.45±0.32	a	AB	++
5	33℃	1.54±0.22	bc	BC	+

*, ** As in Table 1

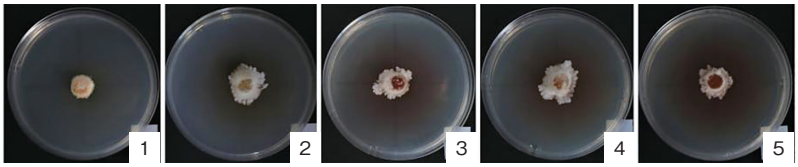

Fig. 6. Effect of temperature on mycelial growth of *Pleurotus dryinus*. (Photos of mycelium culture on the 15th day.)

Table 8. Effect of pH on mycelial growth of *Pleurotus dryinus*

pH		Mycelial growth rate (mm/d)*	Significance levels 0.05	0.01	Mycelial growth vigor (mm/d)**
1	4	0	d	E	+
2	5	2.82±0.41	b	BC	+++
3	6	1.77±0.62	c	D	++
4	7	1.99±0.51	c	CD	++
5	8	2.71±0.62	b	BCD	+++
6	9	4.16±0.47	a	A	+++
7	10	3.28±0.77	b	AB	+++

*, ** As in Table 1

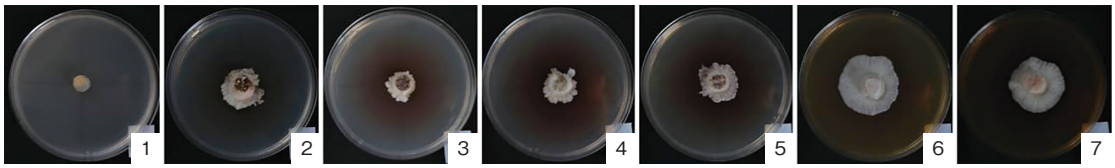

Fig. 7. Effect of pH on mycelial growth of *Pleurotus dryinus*. (Photos of mycelium culture on the 15th day.)

Table 9. Effect of moisture content on mycelial growth of *Pleurotus dryinus*

moisture content		Mycelial growth rate (mm/d)*	Significance levels 0.05	0.01	Mycelial growth vigor (mm/d)**
1	50%	2.09±0.17	ab	A	+++
2	55%	2.15±0.20	a	A	+++
3	60%	2.04±0.26	ab	A	+++
4	65%	1.94±0.38	ab	A	+++
5	70%	1.70±0.41	bc	AB	++
6	75%	1.34±0.56	c	B	+

*, ** As in Table 1

Manufacturing cultural bags

The cultivation medium formulation: sawdust 78%, bran 20%, lime 1%, plaster 1%. Required moisture content of cultivation bags were about 55%. 10 mL liquid spawn per cultural bag was used for inculation (Bonatti *et al.*, 2004).

Spawn running

A clean environment is essential for spawn culture. In this period, the cultivation condition was 20–25°C in darkness and needs appropriate ventilation to keep fresh air. This phase lasts from 25 to 28 days.

Inducement to primordium

The mycelial reached physiological maturity needs 8–10 days after mycelial have been filled with bag. Transfer the culture bags to the fruit chamber. primordium Inducement needs temperature changes, humidity changes and scattered light. This stage takes seven to ten days.

Fruiting period management

The optimal temperature range was 15–25°C, and relative humidity range is 80%–90%. proper ventilation as well as a certain scattered light is essential to fruiting (Fig. 9, Fig. 10).

Harvest

Stop watering before the harvest day. Dig out the original foundation when harvesting (Fig. 11).

In this study, the biological characteristics and domestication cultivation of *Pleurotus dryinus* were studied, the optimal growth medium: carbon source D (+)–maltose, nitrogen source $NaNO_3$, inorganic salt K_2HPO_4 and growth factor straw. The optimum temperature was 22°C and pH was 9.

Wheat and corn were chosen as the material of grain spawns by reason of the better growth vigour than other materials. Using wheat as material, the growth rate, density and germination points of spawn are better than corn, but corn is more economical and practical.

Due to the different culture requirements of strains during growth and development, the physiological characteristics and culture characteristics of different strains are also different. When *P. dryinus* grows on medium, it produces significant chlamydospores. The cultivated

Fig. 8. 1–2. Corn medium. 3. liquid medium.

Fig. 9. 1. The cultural bags. 2–9. Growth phase of *Pleurotus dryinus*.

Fig. 10. 1–7. Growth phase of *Pleurotus dryinus*.

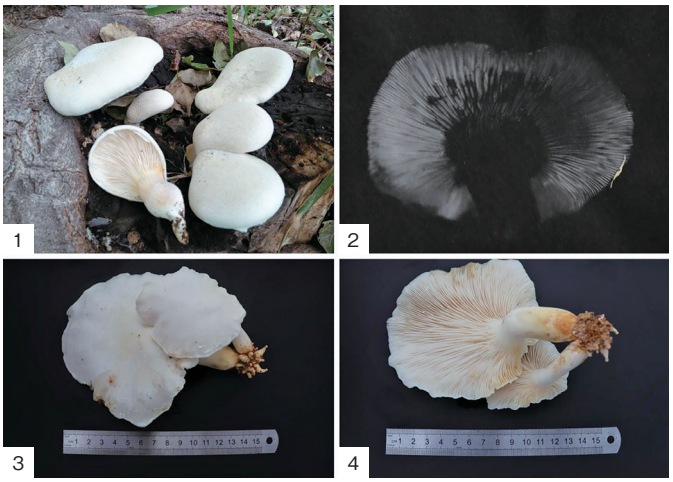

Fig. 11. 1. Wild fruiting body. 2. The spore print 3–4. Cultivated fruiting body.

mushroom spores print is white, which is different from the previous study description. During the phases of fruiting period management, a large amount of chlamydospores are formed on base of the fruit body and culture bags surface, which consumes a part of nutrients. Cleaning culture bags surface after harvested and the use of bottles as cultivation tool can increase the yield of the fruiting bodies.

The pileus was pure white under the condition that humidity was stable and rational. Under certain moisture change, the pileus would dry and crack. The amount of chlamydospores increases when the humidity is insufficient for long time. Under the different mushroom management conditions, the length and width of the stipe, the position of the stipe and the size of the pileus are different.

This paper provides basic research data for development of the use of *P. dryinus*. Follow–up researches of *P. dryinus* can be carried out in these areas of mycelial deep liquid fermentation, formula screening, yield optimization, variety breeding, nutrient composition and pharmacological effects.

AUTHOR CONTRIBUTIONS

W. ZHANG designed the study, performed the mycelial growth observation and wrote the paper. T. BAU and S. OHGA designed the study, supervised the work. Y. OZAKI has made a supervision. All authors assisted in editing the manuscript and approved the final version.

ACKNOWLEGEMENT

Project supported by the Program for Changjiang Scholars and Innovative Research Team in University of Ministry of Education of China (Grant No. IRT15R25).

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