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ZHANG, Yu Xiao
Institute of Mycology/Engineering Research Center of Chinese Ministry of Education for Edible and Medicinal Fungi, Jilin Agricultural University

BAU, Tolgor
Institute of Mycology/Engineering Research Center of Chinese Ministry of Education for Edible and Medicinal Fungi, Jilin Agricultural University

OHGA, Shoji
Institute of Mycology/Engineering Research Center of Chinese Ministry of Education for Edible and Medicinal Fungi, Jilin Agricultural University | Department of Agro-environmental Sciences, Faculty of Agriculture, Kyushu University

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Biological Characteristics and Cultivation of Fruit Body of Wild Edible Mushroom *Auricularia villosula*

Xiao Yu ZHANG[1], Tolgor BAU[1]* and Shoji OHGA[1, 2]*

[1]Institute of Mycology/Engineering Research Center of Chinese Ministry of Education for Edible and Medicinal Fungi, Jilin Agricultural University, Changchun 130118, P. R. China
[2]Department of Agro–environmental Sciences, Faculty of Agriculture, Kyushu University, Sasaguri, Fukuoka 811–2415, Japan
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A special specimen was discovered in Erdos of China, which grown on *Artemisia ordosica*. It is identified as *Auricularia villosula* Malysheva, based on morphological characteristics and polygenic analysis. This is the first time we discovered this species grows on subshrub. Addition, the study of biological characteristics and cultivation has been done for the first time for this species.

For the screening of single optimal factor, each of 3 inorganic salt concentration on from 0.5% to 1.5% at 0.5% interval, each of 8 carbon sources and nitrogen sources and each of 10 growth factors were tested for the mycelia growth rate and mycelia growth potential. As a result, the fungus grew better on maltose, sucrose, fructose as the carbon sources; soybean flour, yeast extract, wheat bran as the nitrogen sources; mushrooms, bean sprouts, potatoes as growth factors and 0.5%K^+, 0.5%PO_4^{3-}, 1%Mg^{2+}. The orthogonal experiments with the above selected three conditions for each of the four factors were then performed and the optimal cultivation conditions were determined. A descending order of the impact for the four factors was growth factors > carbon source > inorganic salt concentration on > nitrogen source and the F–test showed very significant difference among all the four factors. The optimal culture condition for *A. villosula* was the combination of potato juice plus sucrose, soybean powder and 0.5%PO_4^{3-}. Based on the results of orthogonal, temperatures from 15°C to 35°C at 5°C interval, pH at 4 to 8 at 1 interval, as a result, the fungus grew better on 30°C and pH8.

In the domestication experiments of *A. villosula*, the stages of pre–culture spawn, of manufacturing cultural bags, of spawn running, of inducement to primordium, of fruiting period management, of collected periods were studied. The cultivated fruiting bodies showed the appearance identical to the wild ones under optimal conditions.

Key words: biological characteristics, single factor, orthogonal test, domesticated

INTRODUCTION

Auricularia Bull. is a genus in Agaricales Underw (Bulliard 1780), containing 27 species known worldwide, and 15 species were recorded from China (Wu, 2016). Taxonomist attempted to distinguish the species based on the features, such as color, hair length, characteristic of medulla and spores (Lowy, 1952; Kobayasi, 1981; LI Lijia, 1984). However, due to theslightly different between some species and its related, it is considered difficult to be identified. In recently, molecular analysis of sequences, such as ITS, LSU, tef–1, RPB2 *et al*., was widespread used in taxonomy, which makes great contribution to divide different species. (Bandara, 2005; Fan *et al*., 2014; Matheny *et al*., 2007; Malysheva & Bulakh, 2014; Yang *et al*., 2010; Yan *et al*., 1999; Wu *et al*., 2014). It also promotes the study of *Auricularia*.

The Auricularia is well known in Eastation due to its edible and medicinal properties. *Auricularia heimuer* and *Auricularia conera* has been widely cultivated and become a feast on the table. *Auricularia villosula* is also eaten and very popular in Erdos city, Inner Mongolia Autonomous Region, China. But the biological and cultivation Characteristics is not reported. So, this paper conducted a study deep step.

Auricularia villosula Malysheva was described from Russian Far East by Malysheva in 2014, which grown on wood of deciduous trees. In our study, we discovered a special specimen, very similar to *A. villosula*, but with medulla and grown on *Artemisia ordosica* Krasch. (subshrub). So some question emerged. What is this specimen? And What biological characteristics and cultivation Characteristics is? This also the major study in this paper.

MATERIALS AND METHODS

Morphological studies

Specimens were collected from Erdos city, Inner Mongolia Autonomous Region, China. They were dried by hot air oven at 40°C and preserved in the Herbarium Mycology of Jilin Agriculture University (HMJAU), HMJAU37794. Descriptions of species are according to Wu *et al*. (2014). The strain was originally isolated from the fruit body, 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,

[1] Institute of Mycology/Engineering Research Center of Chinese Ministry of Education for Edible and Medicinal Fungi, Jilin Agricultural University, Changchun 130118, P. R. China
[2] Department of Agro–environmental Sciences, Faculty of Agriculture, Kyushu University, Sasaguri, Fukuoka 811–2415, Japan
* Corresponding authors (E–mail: ohga@forest.kyushu–u.ac.jp, junwusuo@126.com)

Table 1. List of species, specimes and locality accession number of sequences used in this study

No	Species	Specimen No.	GenBank ITS	GenBank LSU	GenBank RPB2	Country
1	*Auricularia villosula*	HMJAU37794	• *	•	•	Erdos City, Inner Mongolia Autonomous Region, China
2	*Auricularia villosula*	VLA M–11291	KJ698417	_	KJ698440	Russia, Far East
3	*Auricularia villosula*	LE296422	KJ698418	_	KJ698441	Russia, Far East
4	*Auricularia villosula*	HMJAU2931	•	•	•	Russia, Far East
5	*Auricularia heimuer*	HMJAU7931	•	•	•	Aershan City, Inner Mongolia Autonomous Region, China
6	*Auricularia delicata*	HMJAU2316	•	•	•	Xishuangbanna City, Yunnan Province, China
7	*Auricularia orientalis*	HMJAU6055	•	•	•	Tongliao City, Inner Mongolia Autonomous Region, China
8	*Auricularia americana*	HMJAU21889	•	•	•	Dunhua City, Jilin Province, China
9	*Auricularia heimuer*	Dai 13503	KM396789	KM396840	KP729316	Mudanjiang City, Heilongjiang Province, China
10	*Auricularia heimuer*	Dai 13765	KM396793	KM396844	KP729317	Hailin City, Heilongjiang Province, China
11	*Auricularia americana*	Cui 9887	KM396762	KM396820	KP729306	Yichun City, Heilongjiang Province, China
12	*Auricularia americana*	Dai 13476	KM396764	KM396821	KT152126	AnTu County, Jilin Province, China
13	*Auricularia auricula–judae*	Dai 13210	KM396769	KM396824	KP729312	Lyon, France
14	*Auricularia auricula–judae*	Dai 13549	KM396770	KM396825	KP729313	Lyon, France
15	*Auricularia cornea*	Dai 13547	KX022013	KX022044	KX022073	Danzhou City, Hainan Province, China
16	*Auricularia cornea*	Dai 15336	KX022014	KX022045	KX022074	Nanning City, Guangxi Zhuang Autonomous Region, China
17	*Auricularia delicata*	Dai 16420	KX022025	KX022056	KX022083	Lincang City, Yunnan Province, China
18	*Auricularia delicata*	Dai 11984	KX022017	KX022048	KX022077	Baisha County, Hainan Province, China
19	*Auricularia fuscosuccinea*	FP–102573–SP	KX022027	KX022058	KX022088	State of Louisiana, USA
20	*Auricularia fuscosuccinea*	AG 1548	KX022028	KX022059	KX022089	Sao Paulo, Brazil
21	*Auricularia minutissima*	Dai 14881	KT152104	KT152120	KT152137	Baoding City, Hebei Province, China
22	*Auricularia orientalis*	Dai 14875	KP729270	KP729288	KP729310	Baoding City, Hebei Province, China
23	*Auricularia orientalis*	Dai 1831	KP729271	KP729289	KP729311	Beijing, China
24	*Exidia truncata*	MW 365	AF291279	AF291325	_	Germany

* • means sequenced by us, but did not submit to Genbank

MCCJLAU0343.

Phylogenetic analyses

DNA extraction, PCR are according to Yan and Bau (2017). The ITS, LSU, RPB2 region was respectively amplified with ITS1 and ITS4 (White *et al*., 1990), LR0R and LR7 (Hopple and Vilgalys, 1999), 6F and 7.1R (Matheny *et al*., 2005). 24 sequences were selected for molecular phylogenetic analysis, based on results of BLAST, morphological similarities and referred to the studies of the (Wu *et al*., 2014) and (Malysheva & Bulakh, 2014). 1988 characteristics, including gaps, were used in this study. Sequences were aligned by MUSCLE 3.8.31 (Edgar, 2004). The best model (GTR+I+G) was selected by AIC in Modeltest 3.7 (Posada *et al*., 1998). One million generations were set in Bayesian inference (BI) analyses with best model by MrBayes 3.2.6 (Ronquist & Huelsenbeck, 2003) (Fig. 2).

Culture medium preparation

Medium A: yeast extract 2 g, KH_2PO_4 1 g, $MgSO_4$ 0.5 g, agar 20 g, distilled water 1000 mL; Medium B: glucose 20 g, KH_2PO_4 1 g, $MgSO_4$ 0.5 g, agar 20 g, distilled water 1000 mL; Medium C: glucose 20 g, yeast extract 2 g, VB_1 100 mg, agar 20 g, distilled water 1000 mL; Medium D: glucose 20 g, yeast extract 2 g, agar 20 g, $MgSO_4$ 0.5 g, KH_2PO_4 1 g, distilled water 1000 mL; Medium E: potato 200 g, sugar 20 g, soybean meal 2 g, KH_2PO_4 0.5 g, agar 30 g, distilled water 1000 mL.

Single factor test of carbon and nitrogen source

The PDA medium used for the activated experiment contained (in distilled water): potato 200 g/L, glucose 20 g/L, agar 20 g/L, pH natural. When mycelia had grown all over Petri dishes, it was punched into 5 mm homogeneous pieces at the periphery of colonies by the puncher. These pieces of mycelia were used for the next experiment.

In the test of carbon source, 2% addition level of glucose, sugar, maltose, starch, lactose, CMC–Na, fructose was added individually to the basal medium A. A medium containing no carbon source was used as a control. These medium was autoclaved at 121°C for 30 min. Mycelium was inoculated into the test medium by aseptic operation after autoclaving. Culture dishes were inoculated which then were put to incubator with 25°C. All experiments were performed in sextuple. The diameter of colonies was measured every 24 hours and the mycelial growth was observed until the mycelium was covered with Petri dishes (Shim S M, 2005).

For the nitrogen source test, 2% addition level of yeast extract, peptone, beef extract, carbamide, KNO_3, bran, soybean meal was added individually to the basal medium B. No added served as a control.

Single factor test of inorganic salt and growth factor

On the basis of medium B, an inorganic salt concentration test was carried out. Three concentration gradients (0.5%, 1%, 1.5%) were set for the three inorganic salts (KH_2PO_4, $MgSO_4$, KNO_3).

Growth factors of vitamins (Vitamin C, Vitamin B1, Vitamin E), natural components (potato, bean sprout, malt extract, hay, mushroom, corn) were tested. Apart from out natural components, 0.01 g/L addition level was added individually to the basal medium C. 8 natural components which was severally weighed 20 g/L and boiled the juice with distilled water, filtered was added individually to the basal medium. No added as control (Tie LU, 2007).

Orthogonal test

The orthogonal L_9 (3^4) was used to obtain the optiomal medium in mycelium cultures. Medium D used as the basal medium. For the above four factors(carbon source, nitrogen source, inorganic salt, growth factor) was respectively selected best three levels to carried out the four factors of exercise at three levels orthogonal design (Lu T, 2013).

Single factor test of temperature and pH

Based on the optimal medium of mycelicum cultures were obtained by orthogonal test. To investigate the temperature favorable for the mycelial growth of *A. villosula*, the fungus was incubated for 10 days at 5 different temperature. Medium E used as the basal medium. These medium were put to incubator with 15, 20, 25, 30 and 35°C, individually. pH natural.

To screen pH favorable to the mycelial growth of *A. villosula*, the basal medium E adjust to the range of pH5, 6, 7, 8, 8.5 with 1 M NaOH or HCl was incubated for 10 days at 25°C (Rizal L M, 2015).

Domesticated cultivation experiment

In the domestication experiments of *A. villosula*, the stages of pre–culture spawn, of manufacturing cultural bags, of spawn running, of inducement to primordium, of fruiting period management, of collected periods were studied.

RESULT AND DISCUSSIONS

Taxonomy and Phylogenetic analysis

Auricularia villosula Malysheva, in Malysheva & Bulakh, Nov. sist. Niz. Rast. 48: 174 (2014)

Basidiomata gelatinous when fresh, solitary, or caespitose, rarely caespitose, discoid to auriculate, with even and white margin, projecting up 15–60 mm broad, 0.5–1.0 mm thick. Hymenophore surface usually smooth or rarely folds, pale brown. Upper surface with obviously white abhymenial hairs 49–122 × 4.9–7.3 μm. Shrinkage, solidly, dark brown to black. Internal features. Medulla present, abhymenial hairs, 49–122×4.9–7.3 μm, irregular, hyaline or with slightly fawn, thick–walled with a narrow separated lumen or subsolid, apical tips acute or obtuse, usually tufted. Clamp connections present. Basidia 58–85 × 4.9–6.1 μm, clavate, transversely 3–septate, sterigmata rarely observed. Spores (9.0)11.4–14.8 (16.8) × 4.1–7.8 μm, Q=1.7–2.4(2.8), allantoid, hyaline, thin–walled, smooth, usually guttulate containing one guttules.

Conidia 14.4–19.8(21.7) × (5.4)7.1–10.5 μm, massive, hyaline, septate and slightly constricted at middle or non–septate, non– constricted (Fig. 1).

Habit and habitat: —solitary or caespitose on *Artemisia ordosica* in desert.

Material examined:—CHINA. Inner Mongolia Autonomous Region: Erdos city. 12–sep–2016, Tolgor Bau, *HMJAU 37794*.

Note: Our specimen with medulla and grown on *Artemisia ordosica* Krasch. (subshrub), a little differ to the type of *A. villosula*. But polygenic analysis shows that our specimen and type are group together with high statistical support value (BPP=1) (Fig. 2). It is finally identified as *A. villosula*, based on morphological characteristics and polygenic analysis.

Effect of carton sources and nitrogen on mycelia growth

Mycelium can grow on 8 carbon and nitrogen sources, but sugar and soybean meal were screened as carbon and nitrogen sources suitable for the mycelial growth of *A. villosula* (Table 2, Table3, Fig. 3). After 10 days of incubation, the mycelial growth rate of *A. villosula* recorded 4.30 ± 0.46 mm in sugar and 4.79 ± 0.25 mm in soybean meal, respectively. Among the carbon sources tested, the highest mycelial growth rate was obtained with sugar, followed by fructose and maltose. The rest of carbon sources are in order, lactose, glucose, CK. The

Fig. 1. *Auricularia villosula* (HMJAU37794) 1–2. Habitat and Basidioma; 3. Arrow for medulla; 4. Abhymenial hairs; 5–6. Basidia and Basidioles; 7–8. Basidiospores; 9–10. Conidia. Bars 2=10 mm; 3=200 μm; 4–9=10 μm. Photo by Tolgor Bau.

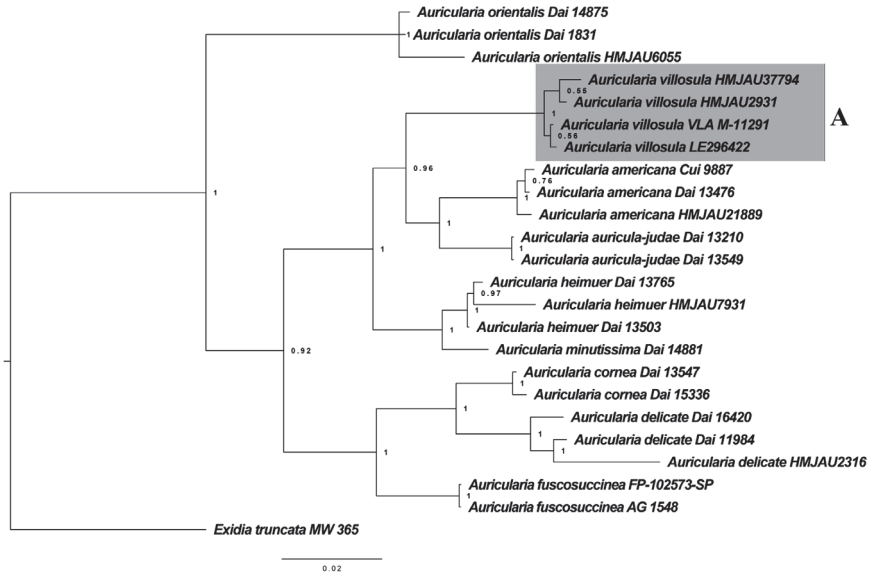

Fig. 2. Bayesian phylogenetic tree inferred from ITS + nLSU + RPB2 sequences.

Table 2. Effect of carbon sources on mycelial growth of *Auricularia villosula*

Carbon source	Mycelial growth rate (mm/d)*	Significance levels		Mycelial growth vigor (mm/d)**
		0.05	0.01	
Sucrose	4.30±0.46	ab	A	+++
Fructose	4.25±0.35	ab	A	+++
Maltose	4.20±0.35	ab	A	+++
Lactose	4.19±0.24	ab	A	+++
Glucose	4.06±0.33	a	A	+++
CK	3.95±0.39	c	B	+++
Soluble Starch	3.72±0.60f	a	A	+++
CMC–Na	1.12±0.32	b	A	++

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

Table 3. Effects of nitrogen sources on mycelial growth of *Auricularia villosula*

Nitrogen source	Mycelial growth rate (mm/d)*	Significance levels		Mycelial growth vigor (mm/d)**
		0.05	0.01	
Soybean meal	4.79±0.25	e	E	++
Bran	4.61±0.31	d	D	++
Yeast extract	4.36±0.32	b	B	+++
CK	3.17±0.15	a	A	++
Potassium nitrate	2.86±0.33	c	C	++
Beef extract	2.30±0.41	f	F	+++
Peptone	1.43±0.11	ab	AB	+++
Carbamide	0.02±0.05	c	C	++

*, ** *As in Table 1*

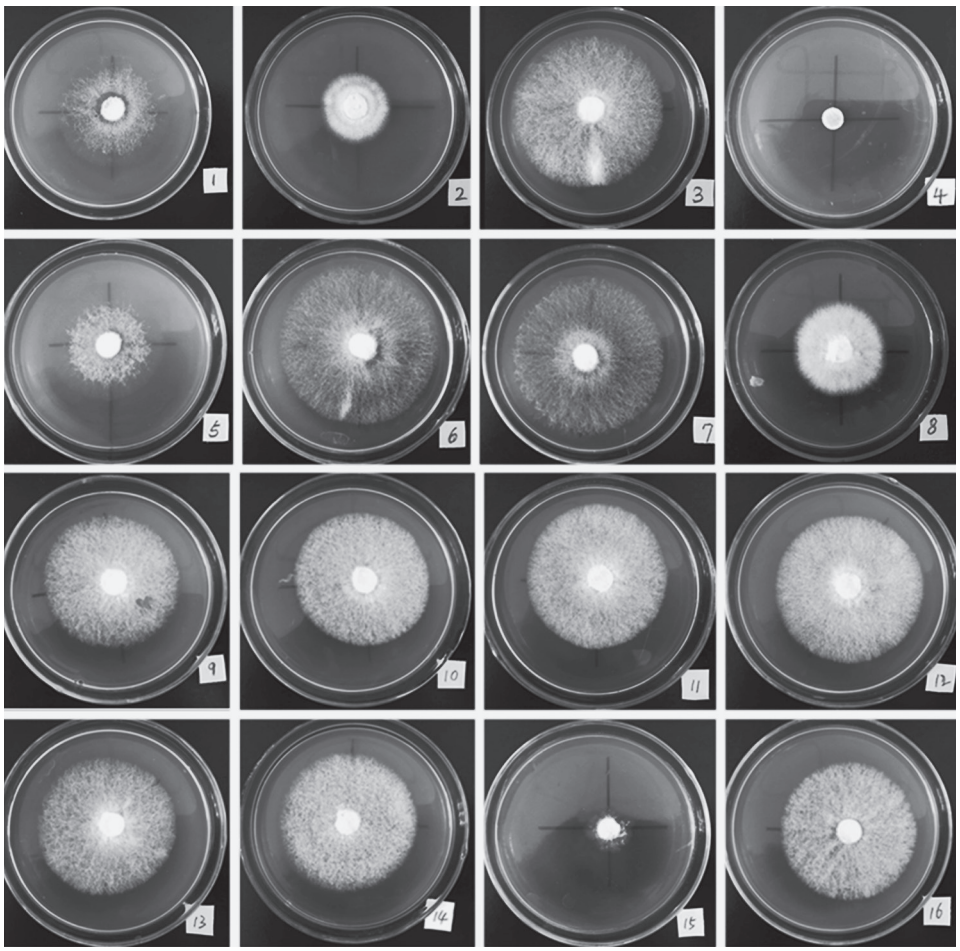

Fig. 3. Colony diameter of *A. villosula* at different Nitrogen source and 1. CK. 2. Peptone. 3. Yeast extract. 4. Carbamide. 5. Potassium nitrate. 6. Bran 7. Soybean meal. 8. Beef extract. 9. Glucose 10. Soluble Starch. 11. Maltose. 12. Sucrose. 13. Fructose.14. Lactose. 15. CMC–Na. 16. CK.

worst are soluble Starch, CMC–Na, these carbon sources were less than control. Apart from outside CMC–Na, the mycelial growth vigor of other carbon sources was more effiectively. Among the nitrogen sources tested, the highest mycelial growth rate was obtained with soybean meal, followed by bran and yeast extract. They are better than control. The Carbamide was the worst, because in the process of autoclaving produced ammonia, mycelium death. From the point of mycelial growth vigor, Yeast extract is more suitable than soybean meal, but also econ-

omy. Therefore, the three carbon (sugar, fructose, maltose) and nitrogen sources (soybean meal, bran, yeast extract) were selected as the optimal condition in the following experiments through comprehensive consideration.

Effect of inorganic salt and growth factor on mycelial growth

The mycelium of *A. villosula* can grow on 10 inorganic salt and growth factor and grow well (Table 4, Table 5, Fig. 4, Fig. 5). 0.5%K^+, 0.5%PO_4^{3-} and 1%Mg^{2+}

Table 4. Effects of inorganic salt concentrationon mycelial growth of *Auricularia villosula*

Inorganic salt concentration	Mycelial growth rate (mm/d)*	Significance levels		Mycelial growth vigor (mm/d)**
		0.05	0.01	
0.5%K^+	4.91±0.37	a	A	+++
0.5%PO_4^{3-}	4.83±0.36	ab	AB	+++
1%Mg^{2+}	4.69±0.25	bc	ABC	+++
CK	4.55±0.19	abcd	ABC	+++
1%K^+	4.51±0.40	abcd	ABC	+++
0.5%Mg^{2+}	4.47±0.40	a	ABC	+++
1%PO_4^{3-}	4.36±0.28	cd	ABC	+++
1.5%Mg^{2+}	4.34±0.16	cd	BC	+++
1.5%PO_4^{3-}	4.31±0.40	cd	BC	+++
1.5%K^+	4.15±0.30	d	C	+++

*, ** *As in Table 1*

Table 5. Effect of Growth factor on mycelial growth of *Auricularia villosula*

Growth factor	Mycelial growth rate (mm/d)*	Significance levels		Mycelial growth vigor (mm/d)**
		0.05	0.01	
Mushroom	4.58±0.50	a	A	+++
Potato	4.40±0.34	ab	AB	+++
Bean sprout	4.31±0.33	ab	AB	+++
Corn	4.22±0.30	abcs	ABC	+++
VE	4.15±0.43	abc	ABC	+++
VC	4.04±0.42	bcd	ABC	+++
VB_1	3.99±0.42	bcd	ABC	+++
CK	3.92±0.32	bcd	ABC	+++
Malt extract	3.74±0.31	cd	BC	+++
Hay	3.60±0.47	d	C	+++

*, ** *As in Table 1*

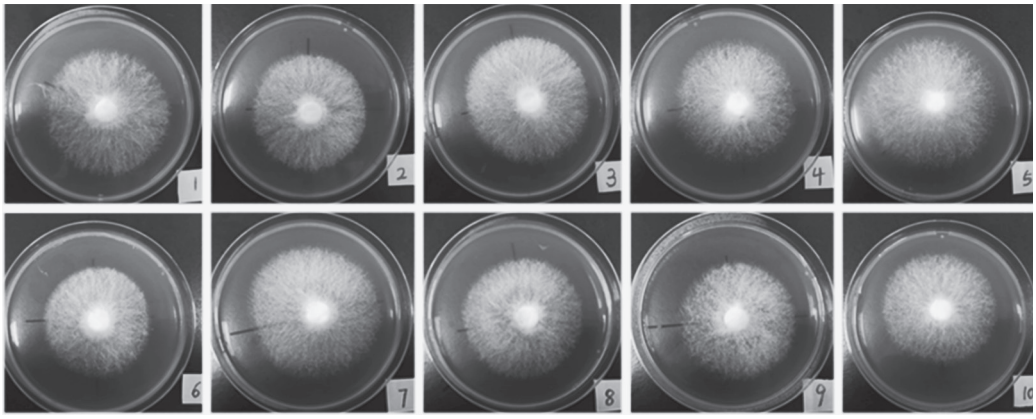

Fig. 4. Colony diameter of *A. villosula* at different Inorganic salt1. 0.5%PO_4^{3-}. 2. 1%PO_4^{3-}. 3. 1.5%PO_4^{3-}. 4. 0.5%Mg^{2+}. 5. 1%Mg^{2+}. 6. 1.5%Mg^{2+} 7. 0.5%K^+. 8. 1%K^+. 9. 1.5%K^+. 10. CK.

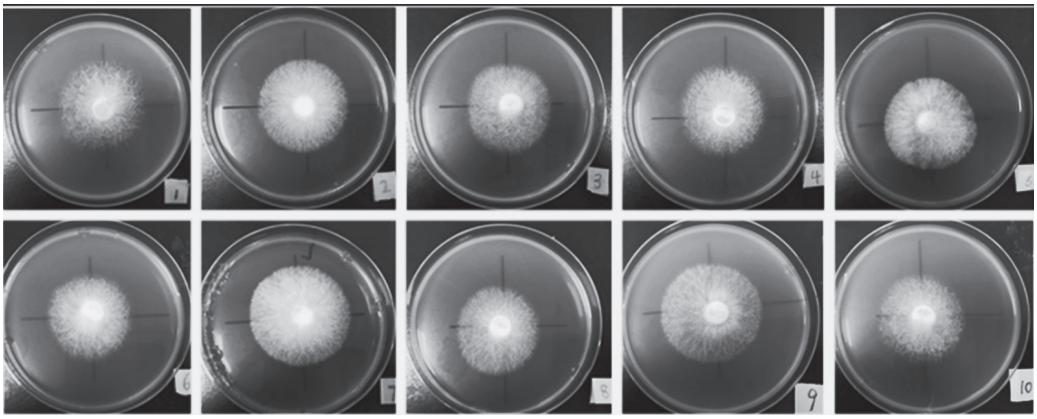

Fig. 5. Colony diameter of *A.villosula* at different Growth factors 1. Potato. 2. Bean sprout. 3. VC. 4. VB. 5. Hay. 6. Malt extract 7. Mushroom. 8. VE. 9. Corn. 10.CK.

Table 6. The results of direct–viewing analysis of mycelial growth

Text Number	Carbon source	Nitrogen source	Inorganic salt concentration	Growth factor	Mycelial growth rate (mm/d)*	Mycelial growth vigor (mm/d)**
1	1Maltose	1soybean meal	1(0.5%K^+)	1Mushroom	2.00	+++
2	1Maltose	2Yeast extract	2(0.5%PO_4^{3-})	2Bean sprout	2.10	+++
3	1Maltose	3Bran	3(1%Mg^{2+})	3Potato	2.31	+++
4	2Sucrose	1soybean meal	2(0.5%PO_4^{3-})	3Potato	2.50	+++
5	2Sucrose	2Yeast extract	3(1%Mg^{2+})	1Mushroom	2.06	+++
6	2Sucrose	3Bran	1(0.5%K^+)	2Bean sprout	2.06	+++
7	3Fructose	1soybean meal	3(1%Mg^{2+})	2Bean sprout	1.92	+++
8	3Fructose	2Yeast extract	1(0.5%K^+)	3Potato	2.13	+++
9	3Fructose	3Bran	2(0.5%PO_4^{3-})	1Mushroom	1.90	+++
T_1	38.50	38.50	37.13	35.75	T=18.98	
T_2	39.75	37.75	39.00	36.50		
T_3	35.63	37.63	37.75	41.63		
X_1	2.14	2.14	2.06	1.99		
X_2	2.21	2.10	2.17	2.03		
X_3	1.98	2.09	2.10	2.31		
R	0.23	0.05	0.10	0.33		

*, ** *As in Table 1*

Table 7. The results of F–test of mycelial growth

Source	TypeⅢSum of squares	*df*	Mean square	*F*	Significance
Carbon source	0.50	2	0.25	3.74	0.0315
Nitrogen source	0.02	2	0.01	0.19	0.8300
Inorganicsalt concentration	0.10	2	0.05	0.76	0.4729
Growth factor	1.14	2	0.57	8.54	0.0007
Error	2.99	45	0.07		
Total	3.95				

was more better than control. Others not only do not benefit, but also inhibit growth. For the growth factors test, mushroom, potato and bean sprout are good candidates for the growth factors compared with other growth factors. Malt extract and hay were no effective from control. Therefore, the three inorganic salts (0.5%K^+, 0.5%PO_4^{3-}, 1%Mg^{2+}) and growth factors (mushroom, potato, bean sprout) were selected as the optimal condition in the following experiments through comprehensive consideration (Zhu Y, 2011).

Effect of orthogonal text on mycelial growth

From the carbon source, nitrogen source, growth factor and inorganic salt concentration in the single factor test, the best three types were selected as the three levels, and the orthogonal analysis of four factors and three levels was established (Table 6, Table 7, Fig. 6). Visual analysis showed that the growth factor. The range is 0.33, followed by carbon source, inorganic salt concentration and nitrogen source. Optimum formulation:

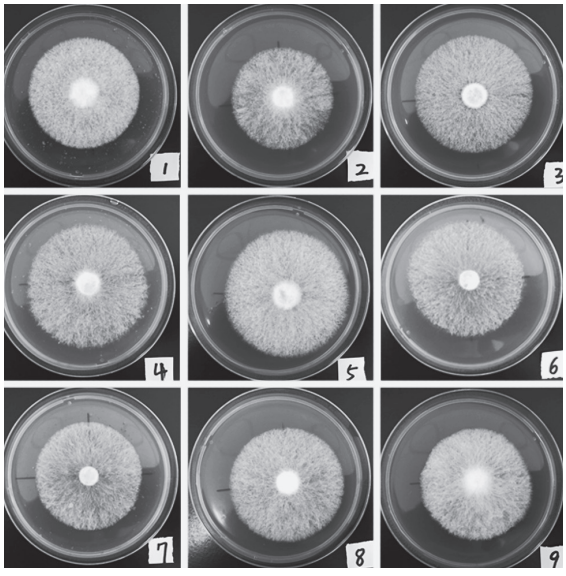

Fig. 6. Colony diameter of *A. villosula* at Orthogonal text 1. Text 1. 2. Text 2. 3. Text 3. 4. Text 4. 5. Text 5. 6. Text 6. 7. Text 7. 8. Text 8. 9. Text 9.

carbon source X2, nitrogen source X1, inorganic salt concentration X2, growth factor X3, fourth group sucrose, soybean meal, 0.5% PO_4^{3-}, potatoes were determined.

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

Effect of temperature and pH on mycelial growth

The temperature experiment was carried out under the optimum formulation selected for orthogonal test (Table 8, Table 9). The mycelium of *A. villosula* could grow at the temperature range of 15°C~35°C, and the mycelial growth rate showed that very significant difference at different temperatures. When the temperature is 30°C, mycelium growth fastest, mycelium was white and dense, strong growth potential, so consider 30°C for the shortest fungus mycelium optimum growth temperature. And when the temperature was 35°C, mycelial growth rate was significantly inhibited by slowing down. The mycelial growth rate of *A. villosula* showed that no significant difference. When pH 8, the mycelium fastest growing, better growth, mycelium thick. And pH 8.5, the mycelium slow down significantly; pH 5, the mycelium growth is weakest. So, it is suitable for alkaline culture.

Table 8. Effecst of temperature on mycelial growth of *Auricularia villosula*

Temperature	Mycelial growth rate (mm/d)*	Significance levels		Mycelial growth vigor (mm/d)**
		0.05	0.01	
15°C	0.83±0.16	a	A	+++
20°C	1.87±0.25	b	B	+++
25°C	2.40±0.78	c	BC	+++
30°C	3.88±0.12	d	C	+++
35°C	2.98±0.42	e	D	+++

Table 9. Effects of pH on mycelial growth of *Auricularia villosula*

pH	Mycelial growth rate (mm/d)*	Significance levels		Mycelial growth vigor (mm/d)**
		0.05	0.01	
5	3.18±0.12	a	A	+++
6	3.28±0.44	a	A	+++
7	3.22±0.59	a	A	+++
8	3.33±0.44	a	A	+++
8.5	3.23±0.53	a	A	+++

Cultivation

Pre–culture spawn

Solid medium: Boiled corn with distilled water, then filled bottle (Jwanny E W, 1995) (Fig. 7).

Liquid medium: 1000 mL fermentation medium, yeasy extract 20 g, glucose 20 g, peptone 10 g, KH_2PO_4 1 g, $MgSO_4$ 0.5 g, and with the conditions of culture: 150 r/min, 7 days at 25°C (Ma J, 2011)(Fig. 8).

They were autoclaved at 121°Cfor 2 h. To inoculated 10 days.

Fig. 7. Mycelial growth of *A. villosula* at Solid medium.

Manufacturing cultural bags

The following recipe of the cultivation medium was used: sawdust 78%, bran 20%, lime 1%, plaster 1%, with water stir evenly and put it in bag (17*30 cm), and autoclaved at 121°C for 2 h. Cultivation bags required water content of about 65%. 10 mL liquid spawn per cultural bag was used for inculation (Bonatti M, 2004).

Fig. 8. Mycelial growth of *A. villosula* at Liquid medium.

Spawn running

Clean environment was essential in order to prevent contamination. The optimal condition for this stage was to keep a steady temperature of 20~25°C and darkness, ventilation for about 15 days (Fig. 9).

Inducement to primordium

After mycelium of culture bag was full up to 10~15 d physiological state of maturity can be moved fruiting induced ear chamber. This time, make the mouth for the bag was required (Fig. 10).

Fig. 9. Spawn running stage of *A. villosula*.

Fruiting period management

The optimal temperature range was 22~30°C, and relative humidity range was 80%~90%, ventilation was needed (B Gizaw 2010) (Fig. 11).

Fig. 10. Inducement to primordium.

Fig. 11. Fruiting priod.

Harvest

To be ear piece of *A. villosula* stretch, the edge began to shrink when the harvest can be. To timely one–time harvest, the whole ears even pull out. The cultivated fruiting bodies showed the appearance identical to the wild ones under optimal condition (Fig. 12).

The optimal growth conditions of the mycelium were determined by the single factor experiment of the carbon source, the nitrogen source, the inorganic salt concentration, the growth factor single factor experiment and the orthogonal test. The optimum growth conditions were as follows: temperature 30°C, pH 8, Carbon source is sucrose, nitrogen source is soybean meal, inorganic salt concentration of 0.5% PO_4^{3-}, growth factor for potato juice. Mycelium best culture temperature of 30°C, need ventilation, dark. Fruiting body growth temperature 21~27°C, air humidity 80%~90%. Ears should be collected before the ejection of spores, harvest ear pieces should be placed in the gauze dry.

So far, *A. heimuer*, *A. conera* and *A. delicata* are have been cultivated widely. *A. heimuer* and *A. conera* is the most extensive cultivation area, the highest yield. Due to the demand in the process of the development of the mycelium is different, so the physiological characteristics of different strains and culture characteristics are different. In this paper, for the mycelium growth of *A. villosul*a , the optimal carbon source is sucrose, however, *A. heimuer* and *A. delicata* is sources too, and the *A. conera* is glucose; the optimal nitrogen source is soybean meal, and *A. heimuer* is yeast extract, *A. delicata* and *A. conera* is beef extract; the optimal tempreture is 30°C, and *A. heimuer* is 30°C, *A. delicata* and *A. conera* is 25°C (Wang jing, 2013; Wu Rengao, 2010; Wang J, 2014).

In this study, the biological characteristics and domestication of *A. villosula* were studied, and the basic research data of biological characteristics was provided for the better utilization of this resource. A follow–up study of *A. villosula* can be carried out in the areas of mycelial liquid deep fermentation, yield optimization, variety breeding and pharmacological active ingredients.

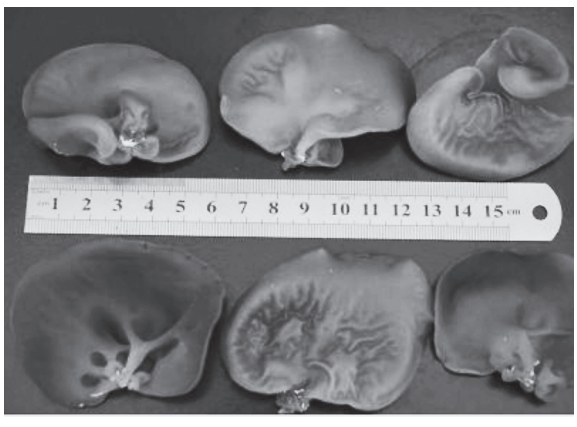

Fig. 12. Fruiting body of cultivation.

AUTHOR CONTRIBUTIONS

X.Y. ZHANG designed the study, performed the microscopic observation, DNA analysis and wrote the paper. T. BAU performed the genetic experiment. T. BAU and S. OHGA designed the study, supervised the work. All authors assisted in editing the manuscript and approved the final version.

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