



## Selection of a highly productive strain of *Pholiota adiposa*

Rong CB<sup>1</sup>, Song S<sup>1</sup>, Niu YR<sup>1</sup>, Xu F<sup>1</sup>, Liu Y<sup>1,2</sup>, Zhao S<sup>1</sup>, Wang SX<sup>1,2</sup>

<sup>1</sup> Institute of Plant and Environment Protection, Beijing Academy of Agriculture and Forestry Sciences, Beijing Engineering Research Center for Edible Mushroom, Beijing 100097, China;

<sup>2</sup> Key Laboratory of Urban Agriculture (North), Ministry of Agriculture, Beijing 100097, China.

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### Abstract

Comparative studies on mushroom strains from various localities are one of the best ways to screen for strains with improved yield and quality. The current study was conducted to evaluate the mycelial growth rate, primordial initiation time, biological efficiency, and nutritional components of four domesticated and one cultivar (Control) strain of *Pholiota adiposa*. Strain JZB2116005 exhibited the highest mycelial growth rate ( $2.56 \pm 0.03$  mm/d), whereas the control strain JZB2116001 was lowest ( $2.34 \pm 0.01$  mm/d). The mycelial colonization time and primordial initiation time of different strains were not consistent with the mycelial growth rates. It took approximately 8–9 days and 11–12 days longer for control strain JZB2116001 to colonize the whole bags and to form primordial, than strain JZB2116005. The highest biological efficiency ( $67.88 \pm 1.33\%$ ) was observed in strain JZB2116005 while the control strain JZB2116001 was worst ( $41.35 \pm 1.72\%$ ). Fruiting bodies of strain JZB2116005 showed better morphological traits and higher chemical contents as compared with the control strain JZB2116001. Therefore, from a commercial point of view, it was necessary to replace strain JZB2116001 with strain JZB2116005 in production.

**Key words** – biological efficiency – cultivation – nutritional components – yield

### Introduction

*Pholiota adiposa* (Fr.) Quel., an edible and medicinal mushroom, is widely distributed on the dead timber piles of poplars, willows, or birches in forest areas in China (Hu et al. 2012). The fruiting body of this mushroom is rich in proteins, essential amino acids, dietary fiber, trace elements, vitamins, and carbohydrates (Hui et al. 2003). Compounds extracted from the fruiting bodies of *P. adiposa* display a variety of important biological activities, such as antitumor (Jiang et al. 2007, Zhang et al. 2009, Hu et al. 2012), antimicrobial (Dulger 2004), anti-HIV-1 (Zhang et al. 2009, Wang et al. 2014a) and antioxidative activities (Ji et al. 2007, Deng et al. 2011, Wang et al. 2014a). Owing to the important nutritional and medicinal properties, cultivation of *P. adiposa* is prevalent worldwide, not only in China but also in several regions of Asia, Europe, and North America (Shimizu et al. 2003).

Recently, numerous reports regarding the comparative cultivation studies of *Hericium* species (Ko et al. 2005), *Flammulina velutipes* (Wang & Wen 2014, Zhang et al. 2015), *Pleurotus* spp. (Zhang et al. 2012, Wang et al. 2014b), *Pleurotus eryngii* (Wu et al. 2011), *Pleurotus ostreatus* (Salmones et al. 2005), and *Tremella fuciformis* (Deng et al. 2014) have been published. However,

the cultivation history of *P. adiposa* is poorly studied, hence, only a few papers are available on the cultural characteristics of *P. adiposa* (Morimoto et al. 1979, Meng et al. 2006, Shen et al. 2009, Li & Qi 2010, Li et al. 2015). There are no comparative studies of different *P. adiposa* strains.

Therefore, the present study was initiated to compare the *P. adiposa* industrial cultivar with 4 new domesticated strains. These strains were evaluated in our preliminary estimation from 12 wild *P. adiposa* strains. Mycelial growth rate (MGR), primordial initiation time (PIT), biological efficiency (BE), and nutritional value of the fruiting bodies were tested to screen an efficient strain with good cultivation traits and nutritional components.

## Materials & Methods

### *Microorganism and spawn preparation*

Twelve *P. adiposa* fruiting bodies collected from different places in Beijing, China, were isolated by tissue culture. The MGR, PIT, and BE were screened in our preliminary estimation. Eight strains were discarded for easily being contaminated by mold or unformed fruiting bodies in cultivation and four strains with relatively good cultivation traits were selected for further study (unpublished data). One cultivar (JZB2116001) with poorly cultivation traits was used as control in this study (Table 1). All the tested strains were deposited in the Beijing Engineering Research Center for Edible Mushroom, Beijing Academy of Agriculture and Forestry Sciences (BAAFS), Beijing, China.

Cultures were maintained on a potato dextrose agar (PDA, 200 g/l of diced potatoes; 20 g/l of glucose; 15 g/l of agar) medium at 25 °C. The spawn preparation was conducted according to the method described by Pant et al. (2006).

### *Substrate preparation, inoculation, and incubation*

The cottonseed hulls, sawdust (*Malus sylvestris*), and wheat bran used in this study for cultivation of *P. adiposa*, were obtained from Beijing Yingliang Agricultural Development Co., Ltd (Beijing, China) and sun-dried. Substrates were prepared with 65% (w/w) water content containing 60% cottonseed hull, 18% sawdust, 15% wheat bran, 5% corn flour, 1% gypsum, and 1% lime and placed in polypropylene bags (17 cm × 33 cm × 0.04 cm) at a packing density of 1,000 g of substrate per bag. A specially designed plastic ring was wrapped at the top end of the bag to form an opening, and an oblong conical plastic rod with a short rope was used to fill the bag from the opening. Then the top plastic ring was covered with a vent cap with microbiological filters. The bags were autoclaved at 121 °C for 120 min. After sterilization, the plastic ring was open carefully and the oblong conical plastic rod was pulled out, a hole was left in the bag, and the spawns were inoculated into the hole at an amount of 2% (w/w) of substrate fresh weight. Two-hundred-fifty replicated polypropylene bags were used and were divided into five replicates for each treatment.

Same substrates were placed in racing tubes for MGR measurements following the method described by Gregori et al. (2008), with a modification of the tube size (30-mm diameter, 260 mm length). Eighty grams of substrate per racing tube were used and sealed with cotton plugs on both sides of the tube. Racing tubes were autoclaved at 121 °C for 120 min. Sterilized tubes were inoculated on one side by spreading the spawn on the substrates at an amount of 2% (w/w) of substrate fresh weight. Five replicates were prepared for each strain.

The inoculated bags and tubes were kept in the spawn running room at 23–25 °C and 50–60% relative humidity (RH) under dark conditions. MGR on substrates filled in racing tubes was measured by the height (mm) of the mycelia in the colonized substrates divided by the incubation time (days). Mycelial colonization time (MCT) (number of days from inoculation to complete colonization of the substrate by the mycelium in the culture bag) and PIT (number of days from inoculation to pinhead fruiting bodies) were recorded.

**Table 1** Sample data of the tested *Pholiota adiposa* strains.

| Strain No. | Origin   | Host   | Acquisition time |
|------------|--|--------|------------------|
| JZB2116001 | Beijing Academy of Agriculture and Forestry Sciences (BAAFS), Beijing, China | —      | —                |
| JZB2116003 | Lingshan, Mentougou district, Beijing, China                                 | Willow | 2009.07.20       |
| JZB2116004 | Lingshan, Mentougou district, Beijing, China                                 | Willow | 2009.07.15       |
| JZB2116005 | Zizhuyuan park, Haidian district, Beijing, China                             | Willow | 2009.09.12       |
| JZB2116007 | Beijing Vegetable Research Center, BAAFS, Beijing, China                     | Willow | 2010.10.09       |

### ***Cropping, harvest, and determination of BE***

After a complete spawn run, the bags were transferred to a refrigerated house to induce an occurrence of primordia and were cultured for 3–5 d at 0–5 °C. Then, the bags were moved to a fruiting chamber that was maintained at  $18 \pm 2$  °C and 80–90% RH with 12 h of illumination (300–600 Lux). Bags were unfolded at the upper parts for cropping. To maintain the desired humidity, the chamber was sprayed intermittently during the growing time.

Fruiting bodies in two flushes were harvested before the caps started to open. The morphological traits of the fruiting bodies, including the pileus diameter and thickness, stipe length and diameter, were measured by digital calipers (Mitutoyo, Japan). The harvested fruiting bodies in each bag were weighed. At the end of the harvesting period, the accumulated data were used to calculate the BE (Yang et al. 2013).

$$\text{BE (\%)} = \text{Weight of fresh mushrooms harvested per bag} / \text{weight of dry weight per bag} \times 100$$

### ***Chemical analysis and statistical analysis***

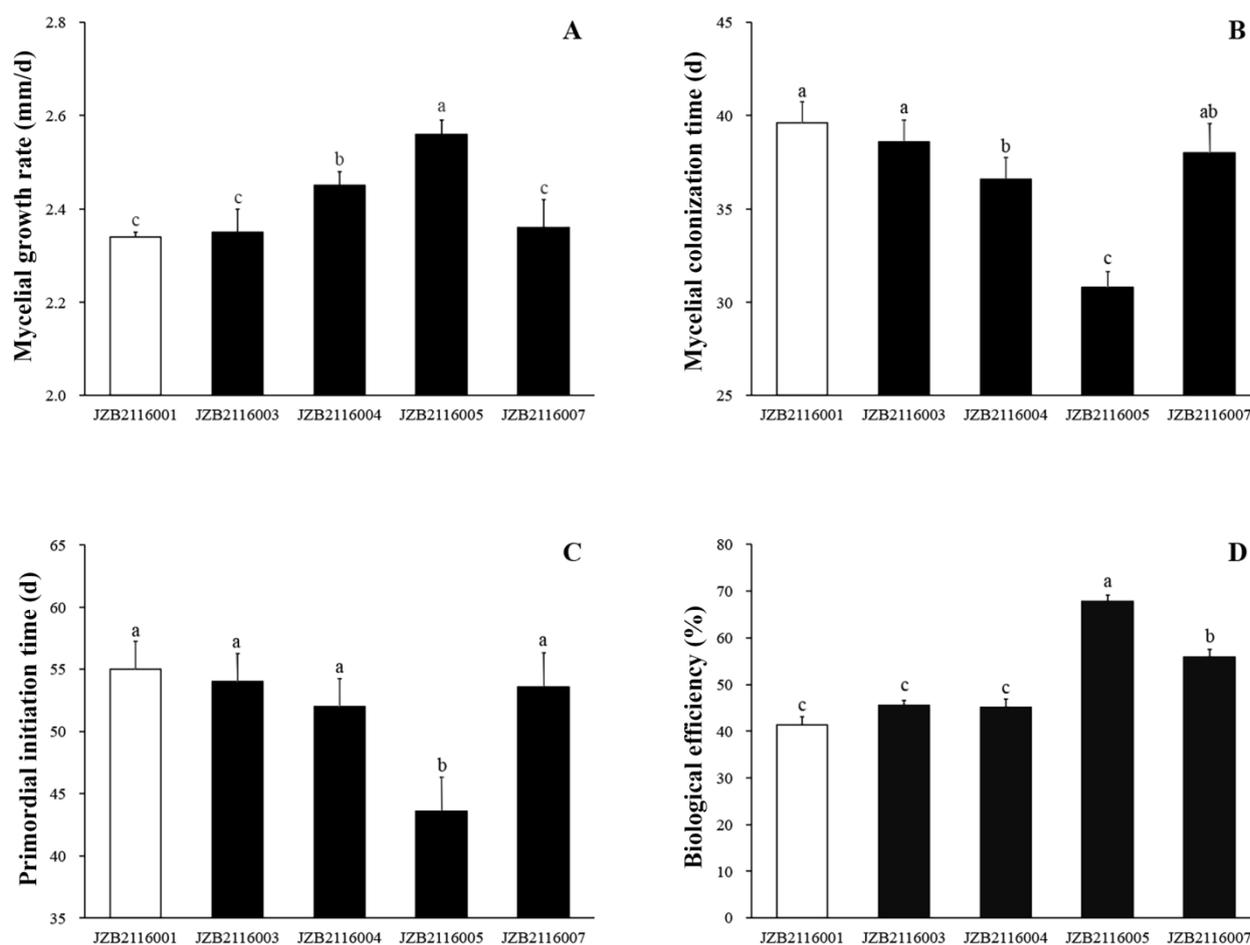
Fruiting bodies of *P. adiposa* were collected randomly from the five tested strains in equal proportions after the first flush. Then they were dried in an oven at 60 °C to a constant weight and kept at 4 °C. The chemical compositions, including moisture, dietary fiber, protein, energy, fat, ash, carbohydrate, and amino acids, of the samples were analyzed following the methods of Wang et al. (2015). All of the above analyses were performed by the PONY Testing International Group (Beijing, China).

Statistical analyses were conducted using the evaluation version of SPSS 20.0 for Windows. Data were obtained from two consecutive harvests, and chemical analyses were subjected to one-way analysis of variance. Differences among the means of eight treatments were assessed using Duncan's multiple range tests at the 95% confidence level.

## **Results**

### ***Mycelial growth and primordial initiation of different strains***

All the tested strains colonized on the substrates containing 60% cottonseed hull, 18% sawdust, 15% wheat bran, 5% corn flour, 1% gypsum, and 1% lime. Out of the tested strains, the highest MGR was observed for strain JZB2116005, followed by strain JZB2116004; the control strain JZB2116001 showed the lowest MGR. The values of the MGR of the above three strains were  $2.56 \pm 0.03$  mm/d,  $2.45 \pm 0.03$  mm/d, and  $2.34 \pm 0.01$  mm/d, respectively. There was a significant difference between strain JZB2116005 and the others with regard to the MGR; no difference was observed among strains JZB2116001, JZB2116003, and JZB2116007 (Figure 1 A). Correspondingly, the MCT and PIT of different strains were inconsistent with the MGR. It took approximately 8–9 d and 11–12 d longer for the control strain JZB2116001 to colonize whole bags and form primordia than strain JZB2116005, respectively (Figure 1 B, C).



**Fig. 1** – Comparison of the mycelial growth rate (MGR) (A), mycelial colonization time (MCT) (B), primordial initiation time (PIT) (C), and biological efficiency (BE) (D) of the tested *Pholiota adiposa* strains.

A, MGR was measured by the height (mm) of the mycelia in the colonized substrates in racing tubes divided by the incubation time (days). B, MCT was measured by number of days from inoculation to complete colonization of the substrate by the mycelium in the culture bag. C, PIT was measured by number of days from inoculation to pinhead fruiting bodies. D, BE (%) = Weight of fresh mushrooms harvested per bag/weight of dry weight per bag  $\times$  100.

**Table 2** Comparison of the morphological traits of the tested *Pholiota adiposa* fruiting bodies<sup>a</sup> (mm) (mean  $\pm$  SD, n= 20).

| Strain No. | Stipe length      | Stipe Diameter    | Pileus thickness  | Pileus diameter    |
|------------|-------------------|-------------------|-------------------|--------------------|
| JZB2116001 | 94.83 $\pm$ 4.83a | 9.67 $\pm$ 1.03b  | 9.17 $\pm$ 0.41a  | 45.67 $\pm$ 3.61a  |
| JZB2116003 | 90.00 $\pm$ 6.28a | 9.60 $\pm$ 0.55b  | 7.40 $\pm$ 1.34b  | 23.60 $\pm$ 5.18bc |
| JZB2116004 | 72.40 $\pm$ 7.13b | 11.40 $\pm$ 2.79b | 6.00 $\pm$ 0.71c  | 18.40 $\pm$ 2.07c  |
| JZB2116005 | 95.80 $\pm$ 8.67a | 11.00 $\pm$ 1.58b | 7.80 $\pm$ 0.84b  | 25.40 $\pm$ 5.41b  |
| JZB2116007 | 65.20 $\pm$ 5.54b | 16.00 $\pm$ 1.87a | 8.20 $\pm$ 2.05ab | 28.60 $\pm$ 4.34b  |

<sup>a</sup>Means in each column followed by the same superscripts are not significantly different at  $P < 0.05$  according to Duncan's multiple range tests.

**Table 3** Comparison of the chemical compositions of the tested *Pholiota adiposa* fruiting bodies<sup>a</sup> (g in 100 g of dry matter, mean  $\pm$  SD, n= 3).

| Parameter         | JZB2116001         | JZB2116003         | JZB2116004         | JZB2116005         | JZB2116007         |
|-------------------|--------------------|--------------------|--------------------|--------------------|--------------------|
| Moisture (g)      | 6.82 $\pm$ 0.05a   | 6.82 $\pm$ 0.04a   | 6.78 $\pm$ 0.03a   | 6.80 $\pm$ 0.06a   | 6.78 $\pm$ 0.09a   |
| Ash (g)           | 6.12 $\pm$ 0.03c   | 6.20 $\pm$ 0.12c   | 7.44 $\pm$ 0.19a   | 7.36 $\pm$ 0.15a   | 6.60 $\pm$ 0.12b   |
| Protein (g)       | 21.90 $\pm$ 0.14c  | 23.20 $\pm$ 0.30b  | 27.20 $\pm$ 0.11a  | 26.60 $\pm$ 0.80a  | 23.20 $\pm$ 0.42b  |
| Fat (g)           | 2.10 $\pm$ 0.01b   | 1.70 $\pm$ 0.05d   | 2.30 $\pm$ 0.01a   | 1.90 $\pm$ 0.03c   | 2.40 $\pm$ 0.13a   |
| Dietary fiber (g) | 30.50 $\pm$ 0.23a  | 31.10 $\pm$ 0.58a  | 30.50 $\pm$ 0.81a  | 27.40 $\pm$ 0.82b  | 27.70 $\pm$ 0.78b  |
| Carbohydrate (g)  | 32.56 $\pm$ 0.25a  | 30.98 $\pm$ 1.01ab | 25.78 $\pm$ 1.13c  | 29.94 $\pm$ 1.84b  | 33.32 $\pm$ 1.28a  |
| Energy (kcal)     | 298.16 $\pm$ 1.53b | 294.64 $\pm$ 1.92c | 293.92 $\pm$ 2.02c | 298.92 $\pm$ 5.05b | 303.83 $\pm$ 2.01a |

<sup>a</sup> Means in each column followed by the same superscripts are not significantly different at P<0.05 according to Duncan's multiple range tests.

**Table 4** Comparison of the amino acid concentration and composition of tested *Pholiota adiposa* fruiting bodies<sup>a</sup> (g in 100 g of dry matter, mean  $\pm$  SD, n= 3).

| Amino acids                | JZB2116001        | JZB2116003        | JZB2116004        | JZB2116005        | JZB2116007        |
|----------------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| Asparagine                 | 1.46 $\pm$ 0.01c  | 1.57 $\pm$ 0.02b  | 1.87 $\pm$ 0.02a  | 1.24 $\pm$ 0.05d  | 1.09 $\pm$ 0.03e  |
| Threonine <sup>b</sup>     | 0.75 $\pm$ 0.01c  | 0.79 $\pm$ 0.03b  | 0.98 $\pm$ 0.01a  | 0.68 $\pm$ 0.03d  | 0.59 $\pm$ 0.02e  |
| Serine                     | 0.78 $\pm$ 0.01c  | 0.87 $\pm$ 0.04b  | 1.01 $\pm$ 0.04a  | 0.66 $\pm$ 0.03d  | 0.58 $\pm$ 0.01e  |
| Glutamic acid              | 3.21 $\pm$ 0.05b  | 3.00 $\pm$ 0.12c  | 4.17 $\pm$ 0.14a  | 2.30 $\pm$ 0.09d  | 2.31 $\pm$ 0.01d  |
| Proline                    | 0.70 $\pm$ 0.03b  | 0.72 $\pm$ 0.01b  | 0.86 $\pm$ 0.01a  | 0.56 $\pm$ 0.05c  | 0.56 $\pm$ 0.01c  |
| Glycine                    | 0.66 $\pm$ 0.01c  | 0.72 $\pm$ 0.01b  | 0.86 $\pm$ 0.01a  | 0.57 $\pm$ 0.02d  | 0.51 $\pm$ 0.02e  |
| Alanine                    | 0.88 $\pm$ 0.01c  | 0.95 $\pm$ 0.01b  | 1.13 $\pm$ 0.01a  | 0.78 $\pm$ 0.03d  | 0.68 $\pm$ 0.02e  |
| Cystine                    | 0.13 $\pm$ 0.01c  | 0.18 $\pm$ 0.00b  | 0.20 $\pm$ 0.00a  | 0.13 $\pm$ 0.01c  | 0.13 $\pm$ 0.01d  |
| Valine <sup>b</sup>        | 0.71 $\pm$ 0.00c  | 0.77 $\pm$ 0.01b  | 0.94 $\pm$ 0.01a  | 0.64 $\pm$ 0.03d  | 0.56 $\pm$ 0.02e  |
| Methionine <sup>b</sup>    | 1.03 $\pm$ 0.02cd | 1.25 $\pm$ 0.08b  | 2.60 $\pm$ 0.16a  | 1.04 $\pm$ 0.05cd | 1.16 $\pm$ 0.04bc |
| Isoleucine <sup>b</sup>    | 0.73 $\pm$ 0.01c  | 0.83 $\pm$ 0.01b  | 0.98 $\pm$ 0.01a  | 0.71 $\pm$ 0.04cd | 0.67 $\pm$ 0.05d  |
| Leucine <sup>b</sup>       | 1.19 $\pm$ 0.01cd | 1.33 $\pm$ 0.05b  | 1.55 $\pm$ 0.02a  | 1.12 $\pm$ 0.06d  | 1.00 $\pm$ 0.09e  |
| Tyrosine                   | 0.40 $\pm$ 0.00c  | 0.47 $\pm$ 0.01b  | 0.53 $\pm$ 0.00a  | 0.37 $\pm$ 0.02d  | 0.33 $\pm$ 0.02e  |
| Phenylalanine <sup>b</sup> | 0.80 $\pm$ 0.01c  | 0.90 $\pm$ 0.03b  | 1.03 $\pm$ 0.03a  | 0.69 $\pm$ 0.03d  | 0.62 $\pm$ 0.03e  |
| Lysine <sup>b</sup>        | 0.82 $\pm$ 0.01d  | 0.97 $\pm$ 0.01b  | 1.11 $\pm$ 0.01a  | 0.88 $\pm$ 0.04c  | 0.80 $\pm$ 0.03d  |
| Histidine                  | 0.33 $\pm$ 0.00c  | 0.38 $\pm$ 0.01b  | 0.43 $\pm$ 0.01a  | 0.33 $\pm$ 0.02c  | 0.31 $\pm$ 0.01d  |
| Tryptophan <sup>b</sup>    | 0.20 $\pm$ 0.00e  | 0.28 $\pm$ 0.00b  | 0.33 $\pm$ 0.01a  | 0.24 $\pm$ 0.00cd | 0.25 $\pm$ 0.01c  |
| Arginine                   | 1.10 $\pm$ 0.02c  | 1.19 $\pm$ 0.02b  | 1.53 $\pm$ 0.02a  | 0.84 $\pm$ 0.04d  | 0.81 $\pm$ 0.04d  |
| EAA <sup>c</sup>           | 6.23 $\pm$ 0.04c  | 7.11 $\pm$ 0.21b  | 9.52 $\pm$ 0.26a  | 5.99 $\pm$ 0.28cd | 5.62 $\pm$ 0.27d  |
| NEAA <sup>d</sup>          | 9.65 $\pm$ 0.12b  | 10.03 $\pm$ 0.24b | 12.59 $\pm$ 0.26a | 7.79 $\pm$ 0.35c  | 7.30 $\pm$ 0.17d  |
| TAA <sup>e</sup>           | 15.88 $\pm$ 0.17c | 17.14 $\pm$ 0.45b | 22.11 $\pm$ 0.51a | 13.78 $\pm$ 0.62d | 12.92 $\pm$ 0.44e |
| EAA/NEAA <sup>f</sup> (%)  | 64.56 $\pm$ 0.37c | 70.88 $\pm$ 0.40b | 75.64 $\pm$ 0.49a | 76.86 $\pm$ 0.13a | 77.01 $\pm$ 1.96a |
| EAA/TAA <sup>g</sup> (%)   | 39.23 $\pm$ 0.14c | 41.48 $\pm$ 0.14b | 43.06 $\pm$ 0.16a | 43.46 $\pm$ 0.04a | 43.50 $\pm$ 0.63a |

<sup>a</sup> Means in each column followed by the same superscripts are not significantly different at P<0.05 according to Duncan's multiple range tests.

<sup>b</sup> Essential amino acids.

<sup>c</sup> EAA, total concentration of essential amino acids.

<sup>d</sup> NEAA, total concentration of non-essential amino acids.

<sup>e</sup> TAA, total concentration of amino acids.

<sup>f</sup> EAA/NEAA, total concentration of essential amino acids/total concentration of non-essential amino acids.

<sup>g</sup> EAA/TAA, total concentration of essential amino acids/total concentration of amino acids.

**Table 5** Comparison of the chemical composition of different origins of the *Pholiota* spp. strains (g in 100 g of dry matter).

| Substrates                                  | Protein     | Fat       | Ash       | Dietary fiber | Carbohydrate | Essential amino acids/Total amino acids (%) | Essential amino acids/ non-Essential amino acids (%) |
|---|-------------|-----------|-----------|---------------|--------------|---|--|
| Current study                               | 21.90–27.20 | 1.70–2.40 | 6.12–7.44 | 27.40–31.10   | 25.78–33.32  | 39.23–43.60                                 | 64.56–77.29  |
| <i>P. squarrosoides</i> (Wang et al. 2014c) | 11.80       | 1.50      | 5.50      | 32.90         | 41.68        | 46.00                                       | 85.19  |
| <i>P. adiposa</i> (Hui et al. 2003)         | 21.64       | 1.22      | 5.88      | —             | —            | 37.87                                       | 60.95  |
| <i>P. nameko</i> (Xiang et al. 2013)        | 24.98       | 3.21      | 6.38      | —             | —            | 28.10*                                      | 39.08*   |

\* Tryptophan, one of the essential amino acids, was undetected.

### Production of *P. adiposa*

BE and morphological traits of the tested *P. adiposa* fruiting bodies were presented in Figure 1 D and Table 2, respectively. The highest BE of fruiting bodies ( $67.88 \pm 1.33\%$ ) was for strain JZB2116005, followed by strain JZB216007, with a BE of  $55.91 \pm 1.65\%$ ; the control strain JZB2116001 showed the lowest BE of fruiting bodies ( $41.35 \pm 1.72\%$ ). There was a significant difference between strain JZB2116005 and the others, and no difference was observed among strains JZB2116001, JZB2116003, and JZB2116004. Strain JZB2116005 displayed the longest stipe length ( $95.80 \pm 8.67$  mm), but no significant difference was observed with strains JZB2116001 and JZB2116003; the values were  $94.83 \pm 4.83$  mm and  $90.00 \pm 6.28$  mm, respectively. Strain JZB2116007 had the shortest stipe length ( $65.20 \pm 5.54$  mm) and the largest stipe diameter ( $16.00 \pm 1.87$  mm). The control strain JZB2116001 showed the largest value in the thickness and diameter of the pileus, with measurements of  $9.17 \pm 0.41$  mm and  $45.67 \pm 3.61$  mm, respectively.

### Chemical contents of *P. adiposa*

Table 3 indicates the chemical compositions of the *P. adiposa* fruiting bodies from different tested strains. Moisture contents of the *P. adiposa* samples were not significantly different among the tested strains. Ash content of the *P. adiposa* samples varied from 6.12 to 7.44 g. The highest protein content was found in strain JZB2116004 ( $27.20 \pm 0.11$  g), followed by strain JZB2116005 ( $26.60 \pm 0.80$  g); the control strain JZB2116001 displayed the lowest protein content ( $21.90 \pm 0.14$  g). Strains JZB2116004 and JZB2116005 showed no significant difference in protein content, but had a significant difference compared with the others. The fat content in strain JZB2116005 was  $1.90 \pm 0.03$  g, which was higher than that of strain ZJB2116003 and lower than the other three tested strains. The highest amount of dietary fiber was found in strain JZB2116003 ( $31.10 \pm 0.58$  g), followed by strains JZB2116001 and JZB2116004, which had an similar dietary fiber amount ( $30.50 \pm 0.23$  g,  $30.50 \pm 0.81$  g); the lowest was in strain JZB2116005, with a dietary fiber amount of  $27.40 \pm 0.82$  g. The carbohydrate content in strain JZB2116005 was  $29.94 \pm 1.84$  g, which was higher than that of strain JZB2116004 ( $25.78 \pm 1.13$  g) and lower than the others. The highest energy was recorded in strain JZB2116007 with  $303.83 \pm 2.10$  kcal, followed by strains JZB2116005 and JZB2116001, with energy values of  $298.92 \pm 5.05$  and  $298.16 \pm 1.53$  kcal, respectively. Strain JZB2116004 demonstrated the lowest energy value ( $293.92 \pm 2.02$  kcal).

Table 4 shows the concentrations of amino acids in different *P. adiposa* fruiting bodies. Interestingly, there was some regularity in the concentrations of the 18 amino acids in tested strains. The highest concentration of the 18 amino acids was in strain JZB2216004, followed by

strain JZB2116003, except for glutamic acid, which showed a lower concentration ( $3.00 \pm 0.12$  g) than that in control strain JZB2116001 ( $3.21 \pm 0.05$  g). Total concentration of amino acids (TAA) in strain JZB2116005 was  $13.78 \pm 0.62$  g, which was higher than that in strain JZB2116007 ( $12.92 \pm 0.44$  g) and lower than the other three strains. Ratios of total concentration of essential amino acids (EAA)/total concentration of non-essential amino acids (NEAA) and EAA/TAA of the five tested strains were demonstrated in Table 4, the highest values were in strain JZB2116007 ( $77.01 \pm 1.96\%$  and  $43.50 \pm 0.63\%$ , respectively), followed by strain JZB2116005 ( $76.86 \pm 0.13\%$  and  $43.46 \pm 0.04\%$ , respectively) and JZB2116004 ( $75.64 \pm 0.49\%$  and  $43.06 \pm 0.16\%$ , respectively). There were no significant differences among these three strains, and the lowest values were in control strain JZB2116001 ( $64.56 \pm 0.37\%$  and  $39.23 \pm 0.14\%$ , respectively).

## Discussion

In mushroom cultivation, the MGRs, PIT, yield, and BE are easily affected by the genotypes of the strains, the origins of the substrates, and the atmospheric conditions, which are typically different. In this study, the fruiting bodies of all the tested *P. adiposa* strains were successfully obtained on the substrates containing 60% cottonseed hull, 18% sawdust, 15% wheat bran, 5% corn flour, 1% gypsum, and 1% lime. Strain JZB2116005 displayed the best characters compared with the others, especially with the control strain JZB2116001. The MGRs of the tested strains were correspond with the BE, but inconsistent with the MCT and PIT (Figure 1). The result of the present study was in agreement with that of Philippoussis (2001) and Baysal (2003) for the cultivation of *P. ostreatus* and Naraian (2009) for the cultivation of *P. florida*. Both of them reported rapid MGR, earlier PIT, and higher yield. However, in our previous comparison studies of tested strains with *P. squarrosoides* strain, *P. squarrosoides* demonstrated the significant difference in MGR, which was approximately 6 d earlier for colonizing the bags than *P. adiposa* JZB2116005, but it needed further 20–22 d for the after-ripening of the mycelia and the BE was 51.29% (Wang *et al.* 2014c), which was lower than that of *P. adiposa* strains JZB2116005 ( $67.88 \pm 1.33\%$ ) and JZB2116007 ( $55.91 \pm 1.65\%$ ) (Figure 1 D) and displayed significant differences among each other (Data not shown). Therefore, strains with the highest MGR should represent a better physiological index that can colonize the whole substrates in a short time and avoid contamination, but the MGR should not be the only index to evaluate the quality of the strains in production. The BE of five tested *P. adiposa* strains was between 41.35 and 67.88% (Figure 1 D); strain JZB2116005 was 1.64-fold higher than the control strain JZB2116001, with a BE of  $67.88 \pm 1.33\%$  and  $41.35 \pm 1.72\%$ , respectively. In addition, among the morphological traits of tested *P. adiposa* fruiting bodies, strain JZB2116005 demonstrated better morphological traits, with the longest stipe length and medium stipe diameter, pileus thickness, and pileus diameter, which were preferred by consumers, in comparison with the other tested strains.

Recently, many mushroom chemical analyses have been reported (Lee *et al.* 2011, Kulshreshtha *et al.* 2013, Wang *et al.* 2014c, Fernandes *et al.* 2015, Wang *et al.* 2015, Xu *et al.* 2015). However, the chemical composition is also easily affected by the strain genotype, substrate origin, and atmospheric conditions, which are usually different. In the present study, the chemical compositions and amino acids of the five tested *P. adiposa* strains were determined (Tables 3, 4), and comparisons of chemical their composition, EAA/TAA ratios and EAA/NEAA ratios of the five tested *P. adiposa* strains with the closely related species in literature are shown in Table 5. The protein contents varied from 21.90 g to 27.20 g and were higher than those of *P. squarrosoides* (11.80 g) (Wang *et al.* 2014c) and *P. adiposa* (21.64 g) (Hui *et al.* 2003). Of the five tested strains, the protein contents of strains JZB2116004 and JZB2116005 were  $27.20 \pm 0.11$  g and  $26.60 \pm 0.80$  g, respectively, which were higher than that of *P. nameko* (24.98 g) (Xiang & Chen 2013). The fat contents (1.70–2.40 g) were higher than those of *P. squarrosoides* (1.50 g) (Wang *et al.* 2014c) and *P. adiposa* (1.22 g) (Hui *et al.* 2003) and lower than that of *P. nameko* (3.21 g) (Xiang & Chen 2013). The ash contents varied from 6.12 g to 7.44 g and were higher than those of *P. squarrosoides* (5.50 g) (Wang *et al.* 2014c) and *P. adiposa* (5.88 g) (Hui *et al.* 2003). Of the five tested strains, the ash content of strains JZB2116004, JZB2116005, and JZB2116007 were  $7.44 \pm 0.19$  g,  $7.36 \pm 0.15$

g, and  $6.60 \pm 0.12$  g, respectively, which were higher than that of *P. nameko* (6.38 g) (Xiang & Chen 2013). The dietary fiber and carbohydrate contents of tested strains were 27.40–31.10 g and 25.78–33.32 g, respectively, which were all lower than those of *P. squarrosa* with values 32.90 g and 41.68 g, respectively (Wang et al. 2014c). The values of EAA/TAA and EAA/NEAA were 39.23–43.60% and 64.56–77.29%, respectively, which were all lower than those of *P. squarrosa* (46.00% and 85.19%, respectively) (Wang et al. 2014c), whereas they were higher than those of *P. adiposa* (37.87% and 60.95%, respectively) (Hui et al. 2003) and *P. nameko* (28.10% and 39.08%, respectively) (Xiang & Chen 2013). In addition, these values of the five tested strains were well above 40% EAA/TAA, and 60% EAA/NEAA is considered to be adequate for an ideal protein food (FAO/WHO, 1973), except for the control strain JZB2116001, with 39.23% EAA/TAA. Therefore, from chemical analyses, it could be concluded that fruiting bodies of strain JZB2116005 demonstrated better nutritional properties compared with the others. And it was necessary to optimize the cultivation formula or environmental factors of strain JZB2116005 for improving its chemical contents in the further research.

In conclusion, this is the first report of a comparative study on *P. adiposa* strains with different origins. From a commercial point of view, strain JZB2116005 has a clear advantage over the control strain JZB2116001 in terms of MGR, MCT, yield, BE, chemical compositions, and amino acid contents, and it is necessary to substitute strain JZB2116001 in production. Our findings will provide the foundation required for upgrading strains of other mushroom species in cultivation.

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