Scientia Horticulturae 234 (2018) 110–115

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Development of a highly productive strain of *Pleurotus tuoliensis* for commercial cultivation by crossbreeding

Shouxian Wang, Shuang Zhao, Zhenxing Huang, Limin Yin, Jie Hu, Jianghong Li, Yu Liu*, Chengbo Rong*

Institute of Plant and Environment Protection, Beijing Academy of Agriculture and Forestry Sciences, Beijing Engineering Research Center for Edible Mushroom, Beijing, 100097, China

ARTICLE INFO

Keywords:
Pleurotus tuoliensis
Bailinggu
Crossbreeding
Biological efficiency
Mushroom

ABSTRACT

Pleurotus tuoliensis, commonly called Bailinggu, is a precious edible mushroom with high nutritional and economic value. However, its low yield, long cultivation period, and non-uniformity of fruiting bodies limit its commercial cultivation. In this study, twenty-five strains were obtained by crossbreeding monokaryotic strains derived from JZB2106012 and JZB2106013, and their genetic and phenotypic characteristics were evaluated. Among these crossbred strains and parental strains, the new hybrid strain C4 displayed the highest biological efficiency, i.e., 67.56%. In addition, C4 showed 90% uniformity of fruiting bodies and had a cultivation period of 92 days, which was two days and thirteen days shorter than those of the parental strains. C4 also had higher hardness, an indicator of quality or shelf life, than the parental strains. C4 was characterized by antagonism tests and inter-simple sequence repeat molecular markers. Genetic differences were observed among parental and crossbred strains. Therefore, the newly developed hybrid strain C4, having a high yield and desirable agronomic traits, might be suitable for commercial cultivation.

1. Introduction

The mushroom *Pleurotus tuoliensis* (Cao et al., 1985; Huang et al., 2011; Kawai et al., 2008; Mou et al., 1987), also known as *P. ferulae*, *P. eryngii* var. *tuoliensis*, or *P. nebrodensis*, is one of the most important commercial edible mushrooms (Zhao et al., 2016). It has been cultivated commercially since 1997 in China (Hu et al., 2010; Zhao et al., 2013), where it is known as Bai-Ling-Gu. It has a white fruiting body with a crisp texture, good taste, and excellent flavor. *P. tuoliensis* has health benefits (Wang et al., 2014; Gao et al., 2018), and compounds extracted from *P. tuoliensis* are rich in polysaccharides, which have potential therapeutic benefits, including immune enhancement (Wang et al., 2014), antitumor activities (Cha et al., 2012), antioxidant activities (Zheng et al., 2004), and cardioprotective effects (Yan et al., 2015). In addition to China, *P. tuoliensis* has become popular in Eastern Asia, including Japan and Korea, owing to its delicious taste and nutritional and medicinal value.

Bailinggu production increased steadily before 2010 in China, and the total production of *P. tuoliensis* reached 323,713 tons in 2010. However, compared with some other edible mushrooms, such as *Lentinula edodes* and *Auricularia auricula-judae*, the production of *P. tuoliensis* is limited. This might be ascribed to the lack of new strains having high yield and quality in recent years. In addition, some biological characteristics, such as a long spawn-running phase, low biological efficiency (BE), and high deformity rate, also limit the commercial cultivation of *P. tuoliensis* (Zhang et al., 2010; Fu et al., 2016).

Therefore, it is important to develop new valuable Bailinggu strains suitable for commercial cultivation. In the present study, a new crossbred strain of Bailinggu with a high BE and high fruiting body uniformity was developed.

2. Materials and methods

2.1. Strains and growth conditions

The strains used in this study are described in Table 1. *P. tuoliensis* Zhongnong No. 1 (CCMSSC 00489, JZB2106013) and JZB2106012 were provided by the China Center for Mushroom Spawn Standards and Control and Beijing Engineering Research Center for Edible Mushrooms, respectively. The strains were cultured and maintained on potato dextrose broth at 25 °C. When required, 1.5% (wt/vol) agar was added to the appropriate medium.

* Corresponding authors.
E-mail addresses: ly6828@sina.com (Y. Liu), woshiboer@163.com (C. Rong).

https://doi.org/10.1016/j.scienta.2018.02.001
Received 22 September 2017; Received in revised form 1 February 2018; Accepted 1 February 2018

Table 1
Strains used in this study.

Strain No.	Description	Source
JZB2106011	Commercial Cultivar	Beijing Jinxin Mushroom Co., Ltd
JZB2106012	Hybrid strain, parent	Beijing Academy of Agriculture and Forestry Sciences
JZB2106013	CCMSSC00489, parent	China Center for Mushroom Spawn Standards and Control
JZB2106014	Wild strain	Beijing Academy of Agriculture and Forestry Sciences
JZB2106015	Wild strain	Beijing Academy of Agriculture and Forestry Sciences
JZB2106016	Hybrid strain	Beijing Academy of Agriculture and Forestry Sciences
JZB2106017	Crossbred strain 4, this study	Beijing Academy of Agriculture and Forestry Sciences
JZB2106018	Crossbred strain 11, this study	Beijing Academy of Agriculture and Forestry Sciences
JZB2106019	Hybrid strain	Beijing Academy of Agriculture and Forestry Sciences
JZB2106020	Hybrid strain	Beijing Academy of Agriculture and Forestry Sciences
JZB2106021	Hybrid strain	Beijing Academy of Agriculture and Forestry Sciences

2.2. Single-spore isolation

Fruiting bodies were placed in Erlenmeyer flasks containing 100 ml of sterilized water to generate the spore suspension. The spore suspensions were subjected to sequential gradient dilution with sterilized water until the concentration was approximately 1×10^3 spores/ml. One hundred microliters of spore suspension was spread onto potato dextrose agar (PDA) plates and incubated at 25 °C for ten days for spore germination. On the basis of the absence of clamp connections on mycelia by microscopy (400 × magnification), we ascertained whether the mycelium originating from a monospore was a monokaryon.

2.3. Pairings between single-spore isolates

Inter-strain pairing experiments were performed using random single-spore isolates (SSIs) derived from the strains JZB2106012 and JZB2106013. Mycelia of two different SSIs were paired on PDA plates and incubated at 25 °C. Mycelium fragments (5 mm) were taken from the contact zone between the paired colonies and individually transferred to PDA plates for further incubation. When the colonies grew to 1.0–1.5 cm in radius, the mycelia were monitored using a microscope to identify dikaryotic hybrids identified by clamp connections.

2.4. Antagonistic activity tests

Antagonistic activity assays were performed as previously described, with minor modifications (Xiang et al., 2016). Briefly, two parental strains and the putative hybrids were co-cultured at 25 °C in 9-cm PDA plates; all mycelial fragments were placed 2 cm apart. Somatic incompatibility reactions were observed after incubation for 10 days.

2.5. Fruiting and cultivation methods

Crossbred strains and parental strains were cultivated for fruiting tests. The raw substrates were 39% cottonseed hull, 6% sawdust, 6% cottonseed extract, 17% corncob, 23% wheat bran, 5% corn flour, 3% lime, and 1% light calcium carbonate. The water content was adjusted to approximately 62%. The mixed substrates were placed in polypropylene bags at 1350 g per bag. The bags were autoclaved at 121 °C for 70 min. The spawns were inoculated in the bags at 2% (w/w) of the substrate wet weight. The inoculated bags were incubated at 20–22 °C in dark conditions. The bags were colonized completely by mycelia at the 40th day after inoculation. Incubation was continued for 30 days. Mycelial differentiation was induced by stimulation at 4 °C for 8 days. The bags were then transferred to a fruiting chamber that was maintained at 12 °C and a relative humidity of 85–90% for 20–30 days to harvest the fruiting bodies. Each strain was subjected to three treatments, 200 bags per treatment. For industrial production, six thousand bags per strain were prepared. The agronomic traits of the fruiting bodies (thirty randomly selected fruiting bodies of each strain), including the pileus length, pileus width, pileus height, stipe length, hardness, and weight of fruiting bodies, were measured. The hardness measurements of the pileus of Bailinggu were performed as previously described with minor modifications (Kim et al., 2013). Hardness of the inside portions of the pileus was measured using a 6 mm cylindrical probe. The number of deformed fruiting bodies was calculated for each treatment group with 200 fruiting bodies. Fruiting body uniformity % = (1 - number of deformed fruiting bodies/200) × 100. At the end of the harvesting period, the BE was calculated based on the following formula: BE (%) = weight of fresh mushrooms harvested per bag/dry weight per bag × 100.

2.6. Inter-simple sequence repeat analysis

Twenty-three random primers were selected for the inter-simple sequence repeat (ISSR) analysis. ISSR amplification was performed in 20-μl reaction volumes containing 0.5 U of *Taq* DNA polymerase, 2.5 mmol/l $MgCl_2$, 0.2 mmol/l dNTPs (Takara, Shiga, Japan), 0.75 μmol/l primer, and 50 ng of template DNA. Amplification conditions were as follows: 94 °C for 5 min; 35 cycles of 94 °C for 30 s, 41 °C for 45 s, and 72 °C for 90 s; and a final extension for 7 min at 72 °C. Polymorphic DNA bands were recorded as present (1) or absent (0). A dendrogram was constructed based on the coefficients obtained by the unweighted pair-group method with arithmetic mean (UPGMA) based on each simple matching matrix (Yin et al., 2014).

2.7. Statistical analysis

All data were statistically analyzed using SPSS software PASW Statistics version 18. All data were in triplicate. Accession differences were determined by Duncan's multiple range test (DMRT). Statistical differences were considered at the 5.0% level of significance ($P < .05$).

3. Results

3.1. Characteristics of crossbred strains

Interbreeding was conducted by mating thirty-two monokaryons isolated from the parental strains JZB2106012 and JZB2106013. Each of the sixteen monokaryotic mycelia of JZB2106012 was mated with the sixteen monokaryons of JZB2106013, resulting in a total of two hundred fifty-six matings. Crossbred strains were screened for clamp connections within the contact zone using a microscope. The presence of clamp connections indicates the formation of a dikaryon (Sou et al., 2013). In total, sixty-eight strains exhibited clamp connections; the mating frequency was 26.6%. The fifty-six putative hybrids showed strong somatic incompatibility reactions with both parental strains. Twenty-five crossbred strains were fully grown on PDA within 10 days; the mycelial growth rate (MGR) of these 25 strains was 9.15–12.84 mm/day on PDA at 25 °C. The mycelial growth rate of the 25 crossbred strains was measured on sawdust medium (Fig. 1). The MGR of parental strain JZB2106013 was 2.32 ± 0.09 mm/day, which