The Cultivars of *Pleurotus nebrodensis* in China

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Abstract: *Pleurotus nebrodensis* (Bailinggu in Chinese) was first cultivated successfully in 1987, after long being considered a healthy food for the local people of Xinjiang, China. The commercially cultivated spawn was isolated from the tissue of wild mushrooms in Xinjiang. Identification was carried out by differentiation of culture characteristics, agronomic characteristics and IGS2-RFLP diagrams for the tested strains collected from the commercial areas. The results show a diversity of commercial strains in China. They can be divided into two groups based on fruiting body morphology: i.e. funnel-shaped or palm. The palm group contained three strains. They are clearly differentiated in terms of morphology as well as culture characteristics, agronomy and IGS2-RFLP DNA fingerprinting.

Key words: *Pleurotus nebrodensis*, cultivars, fruit body morphology, agronomy, IGS2-RFLP, differentiation

1 Introduction

Bailinggu was firstly recorded as *Agaricus nebrodensis* Lanze,[1] but later placed in the genus *Pleurotus* and named *P. nebrodensis*.[1, 2] It is distributed widely in Sicily in Italy, and has been discovered and collected as food in Israel, Syria and France. In China, it can be found on the basic stem or root of *Ferula sinkiangensis* in Tianshan, Tuoli, Aertai, Tacheng in Xinjiang, and was identified as *P. nebrodensis* by the Chinese mycological taxonomist, Xiaolan Mao.[3] It was taken for food by indigenous people and called "Tianshan holy mushroom" [4] for its rich nutrition and healthy function. The wild mushroom was eventually cultivated in 1987.[4] *P. nebrodensis* is now widely cultivated and has become the mushrooming mushroom in Xingjiang, Beijing, Fujian, Henan, Shanxi and Shandong in China. In 2003, output was over 10,000 tons according to statistics from the China Edible Fungi Association.

Various natural morphological characteristics appear in *P. nebrodensis* fruit bodies. The pileus is white or ivory, depressed, palmate, fan-shaped, spoon-shaped, or funnel-shaped, with radiate, small dim stripes or squama; lamellae are decurrent, and light salmon in colour. Usually, the end of lamella is anastomose; the stipe is lateral, 1-7cm long and 1.6-5cm in diameter. The basidiospore is oval or long-oval, 11-15.96 × 5-7.78μm.

2 Materials and Methods

Seventeen strains were collected from the main cultivation areas for *P. nebrodensis* in Xinjiang, Beijing, Fujian, Henan, Shanxi, Shandong and elsewhere. Results from antagonistic testing show that they belong to four different cultivars. The four cultivars were studied on the basis of morphology, agronomical and cultural characteristics, and IGS2-RFLP.

2.1 Agronomical characteristics

The substrate consisted of 85% cottonseed hull, 14% wheat bran and 1% gypsum. The temperature was below 22°C for mycelia running and 10-20°C for fruiting. Morphological characteristics were observed.

2.2 Culture characteristics

Cultures were grown in Petri dishes (90mm) on Difco™ Potato Dextrose Agar (PDA) medium in the dark at 5°C, 10°C, 15°C, 20°C, 25°C, 30°C and 35°C, and growth was determined by measuring the diameter of colony.

2.3 IGS2-RFLP

Total DNA was extracted from mycelia using the method of Lee and Taylor,[5], digested with restriction enzymes *Bsh*1236 I, *Bsu*R I, *Hin*6 I and *Rsa* I, then subjected to electrophoresis in 2% (w/v) agarose gel and observed using the Gel Electriphoresis Analyses System after staining with ethidium bromide.

3 Results

3.1 Agronomic characteristics

Fruit body morphology is clearly different among the cultivars, which were divided into two groups: palmate and funnel-shaped on the basis of this characteristic (Figure 1). In the palmate group, lateral stipe morphology can be used to differentiate further among the cultivars. The cultivars also varied according to temperature stimulation, reaction to light, and different spawn running periods for fruiting in cultivation (Table 1).

Figure1. Fruit body of *P. nebrodensis*
Right: funnel-shaped; left: palmate.

Table 1. Cultivation characteristics of *P. nebrodensis* cultivars

Cultivar (ACCC)	Degree of stipe adnation	Concave surface	Optimum temp. for fruiting (°C)	Running period for fruiting (days)	Reaction to light	Low temp. stimulation
51060	Almost central	Yes	15 - 20°C	60	Fruiting >300Lux	No
50869	Lateral.	No	12 - 16°C	100	Fruiting >600Lux	10-15°C
51452	Eccentric		14 - 17°C	90	Fruiting >600Lux	10-12°C
51453	Almost lateral.	No	14 - 17°C	80	Fruiting in the dark	10-12°C

3.2 Culture characteristics

The mycelium is white and the colony is stretched, uniform and sparse (Figure 2). The hyphae of *P. nebrodensis*

have clamp connections. The optimum temperature range for mycelial growth is 20-30°C: the mycelium die after exposure to 35°C for 2hr. The growth rate at a set temperature varies among the cultivars. The optimum temperature is different between funnel-shaped and palmate cultivars (Table 2): i.e. 25°C for the former and 25-30°C for the latter. Incompatible or antagonistic reactions occur between the cultivars, and appear as a visible pigment line at the margin of two colonies (Figure 3).

Figure 2. Colony of *P. nebrodensis* cultivar

Figure.3 Antagonism between two *P. nebrodensis* cultivars

Table 2. Mycelium growth rate of *P. nebrodensis* cultivars

Cultivar (ACCC)	Growth Rate (mm/day)		
	20°C	25°C	30°C
51060	5.1	5.9	2.0
50869	9.5	11.8	10.9
51452	7.2	10.2	8.2
51453	8.5	10.0	8.9

3.3 IGS2-RFLP fingerprint

The IGS2-RFLP fingerprint is distinct among the different cultivars after DNA digestion with restriction enzymes *Bsh*1236I,, *Bsu*RI, *Hin*6I and *Rsa*I (Figure 4) indicating that the cutting loci are different due to differences in the DNA sequence of the IGS2 region of the cultivars. The difference trends were consistent with differences in culture and cultivation characteristics.

4 Analysis and Discussion

All cultivars of Bailingu cultivated China were from Xinjiang where a lot of fruit bodies occur in the wild. The wild fruit bodies were isolated and selected, and finally several became commercial cultivars. During the past 20 years, breeders and growers have made efforts to breed better cultivars by seeking monokaryons between the wild strains or hybrids. All these efforts enlarge the diversity of cultivar germplasm. There was only one funnel-shaped cultivar, ACCC 50160, before 1994 according to records and tests for collected cultures (Table 1). The palmate, ACCC50869, become a commercial cultivar in 1995 and, later, further palmate cutivars (i.e. ACCC51452 and ACCC51453) were selected and used in production. We believe that more new cultivars of Bailingu, *P. nebrodensis*, will appear in China.

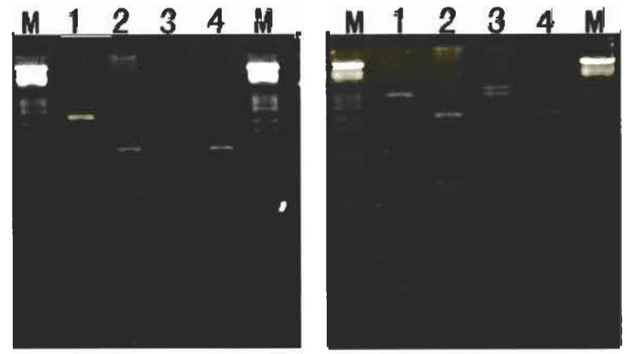

Figure 3. IGS2-RFLP profiles of the tested strains
Left: digested with *Bsu*R I; Right: digested by *Rsa* I. M = marker (λDNA/*Hind* III + *Eco*R I)

At present, 17 cultures collected from Beijing, Shanxi, Shandong, Henan, Xinjiang and Fujian were divided into two groups, funnel-shaped and palmate, based on fruit body morphology. This study shows that four cultivars are distributed across the two groups on the basis of culture characteristics, fruiting test and IGS2-RFLP analysis of rDNA. Three palmate cultivars, ACCC50869, ACCC51452 and ACCC51453, are popularly used in commercial production. Among them, ACCC50869 is the most popular.

Bailinggu grows on the root and basic stem of *Ferula sinkiangensis* in Xinjiang and, as stated above, the diversity of morphology is very abundant. The incompatible-antagonistic reaction was clearly apparent among cultivars, though there was only little differentiation among the palmate ones in terms of morphology. Based on all the characters examined, the distinction between cultivars was very clear; e.g. the period of spawn running, reaction to temperature and light. DNA differences are more distinct and more convenient for discrimination of the cultivars. In this study, the cultivars were analyzed by IGS1-RFLP, and the result indicated that there were no differences. However, analysis by IGS2-RFLP revealed clear distinctions among the cultivars. The results are stable and reproducible, and support differences in mycelium and fruit body characteristics. This suggests that IGS2-RFLP analysis could be used to identify or discriminate cultivars in order to protect variety rights. In other words, the DNA fingerprint diagram of IGS2-RFLP could be used as a molecular identification card for *P. nebrodensis* cultivars or varieties.

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