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FULL PAPER

Genshiro Kawai · Katsuhiko Babasaki · Hitoshi Neda

Taxonomic position of a Chinese *Pleurotus* "Bai-Ling-Gu": it belongs to *Pleurotus eryngii* (DC.: Fr.) Quél. and evolved independently in China

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Abstract The Chinese Bai-Ling-Gu is a mushroom named *Pleurotus eryngii* var. *tuoliensis* C.J. Mou. This species has been identified as *P. nebrodensis* or *P. eryngii* var. *nebrodensis*. We examined its taxonomic position by analysis of mating, cultivation, and rDNA sequences, and concluded as follows. (1) Bai-Ling-Gu mated with *P. eryngii* var. *eryngii*, and the F_1 and F_2 formed fruit bodies. (2) Bai-Ling-Gu mated with *P. eryngii* var. *ferulae*, and the F_1 formed fruit bodies. (3) In the di-mon mating test, *P. eryngii* var. *nebrodensis* from Sicily mated with monokaryons of *P. eryngii* var. *eryngii* but mated hardly at all with those of Bai-Ling-Gu and *P. eryngii* var. *ferulae*. The di-mon mating pattern of Bai-Ling-Gu resembled those of *P. eryngii* var. *ferulae*. (4) The partial sequences of rDNA ITS1 and IGS1 from the epitype of *P. nebrodensis* were identical with those from *P. eryngii* var. *nebrodensis* from Sicily but differed from those from Bai-Ling-Gu. (5) The strains of *P. eryngii* var. *eryngii* and *P. eryngii* var. *ferulae* were in a group, the strains of *P. eryngii* var. *nebrodensis* from Sicily were in another group, and the strains of Bai-Ling-Gu were in the other group in both the phylogenetic trees based on the ITS1 and the IGS1 sequences. These results led to the conclusion that Bai-Ling-Gu is a variety of *P. eryngii* and evolved independently in China. It is satisfactory to identify Bai-Ling-Gu with *P. eryngii* var. *tuoliensis* C.J. Mou.

Key words Bai-Ling-Gu · *Pleurotus eryngii* var. *tuoliensis* · *Pleurotus nebrodensis* · rDNA intergenic spacer (IGS) · rDNA internal transcribed spacer (ITS)

G. Kawai (✉)[1]
Asahimatsu Food Research Laboratory, Nagano, Japan

K. Babasaki · H. Neda
Forestry and Forest Products Research Institute, Ibaraki, Japan

Present address:
[1]1-32-17 Shibamata, Katsushika-ku, Tokyo 125-0052, Japan
Tel. +81-3-5699-2251; Fax +81-3-5699-2251
e-mail: gen999@nifty.com

Introduction

A Chinese *Pleurotus*, "Bai-Ling-Gu," is a choice edible mushroom in the Chinese market and is beginning to be imported and cultivated in Japan. Bai-Ling-Gu is the Chinese trade name for the mushroom (Mao 2005). The whole fruit body is white. The cap is 4–13 cm broad, convex, or flat with incurved margin and thick flesh. The gills are decurrent, and the stem is adnate or subcentral and very short. The spores are ellipsoidal, 10.8–14 × 4.8–6 μm in size. *Pleurotus nebrodensis* (Inzenga) Quél. has been usually used as the scientific name (Mao 2000). *Pleurotus eryngii* (DC.: Fr.) Quél. var. *nebrodensis* Inzenga (Huang 1998) and *P. eryngii* var. *tuoliensis* C.J. Mou (Mou et al. 1987) were also used. Bao et al. concluded that *P. nebrodensis* from China and *P. eryngii* from China were independent, incompatible species (biological species) from mon-mon mating tests (Bao et al. 2004a) and phylogenetic analysis based on polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) of the partial 26S rDNA (Bao et al. 2004b). Zhang et al. (2006) carried out mating tests, rDNA internal transcribed spacer (ITS) sequence analysis, rDNA intergenic spacer (IGS)1-restriction fragment length polymorphism (IGS1-RFLP), IGS1 sequence analysis, and IGS2-RFLP analysis of 17 morphologically different *Pleurotus* mushrooms collected on *Ferula sinkiangensis* K.M. Shen. The isolates were divided into two groups identical to *P. eryngii* var. *ferulae* and *P. nebrodensis* of China based on mating tests and ITS sequence analysis. Single spore isolates from these two groups were incompatible, but those from *P. eryngii* var. *ferulae* and *P. eryngii* of Italy were 56% compatible.

There are three kinds of *P. eryngii* sensu lato in China: *P. eryngii* var. *eryngii* or *P. eryngii*, *P. eryngii* var. *ferulae* or *P. ferulae* (A-Wei-Mo), and *P. eryngii* var. *nebrodensis* or *P. nebrodensis* (Bai-Ling-Gu) (Huang 1998; Mao 2000). The mushroom of *P. eryngii* has a gray to gray-brown cap with a diameter of 4–8 cm and white stem of diameter 0.8–4 cm and length 3.5–15 cm. The host plants are *Eryngium* spp. A-Wei-Mo has a gray-brown to dull white

big cap, diameter 5–15 cm, and a white to dull white stem, of diameter 1–3 cm and length 2–7 cm. The host plants are *Ferula* spp. Bai-Ling-Gu has a pure white cap of diameter 4–13 cm and a pure white stem of diameter 1–3 cm and length 3–4 cm. The host plants are also *Ferula* spp.

Pleurotus nebrodensis was first described as a rare taxon of Sicilian (Italy) mycotaxon (Inzenga 1863). *P. nebrodensis* is usually found on *Cachrys ferulacea* (L.) Calest. and is mostly located in Sicily (Venturella 2000). The basidiospores in *P. nebrodensis* are widely cylindrical, 15–18 × 6–8 μm (Venturella 2000). Host plants and spore size differ between the Sicilian and Chinese mushrooms. The host plants of Chinese *P. nebrodensis* (Bai-Ling-Gu) are *F. sinkiangensis* K.M. Shen (Zhang et al. 2006) and *F. ferulaeoides* (Steud.) Korov. (Mou et al. 1987). The basidiospores in Chinese *P. nebrodensis* (Bai-Ling-Gu) are ellipsoidal, 10.8–14 × 4.8–6 μm (Mao 2005).

Hilber (1982) studied the *Pleurotus eryngii* complex and reported that "*eryngii*," "*ferulae*," and "*nebrodensis*" are intercompatible, being the same species but different varieties. They were recommended to be designated as *P. eryngii* var. *eryngii*, *P. eryngii* var. *ferulae*, and *P. eringii* var. *nebrodensis*, respectively. The host plants of "*eryngii*" are *Eryngium campestre* L., *E. maritimum* L., and *Carlina gummifera* Less; those of "*ferulae*" are *Ferula* sp. and *Ferulago* sp.; and those of "*nebrodensi*s" are *Laserpitium latifolium* L., *L. siler* L., and *C. ferulacea*.

The *Pleurotus eryngii* complex was often discriminated by their host plants, and it was supported by enzyme polymorphism (Catherine and Dubayle 1983), the compatibility test (Cailleux et al. 1981; Zerbakis and Balis 1996), and random amplified polymorphic DNA (RAPD) analysis and enzyme profiles (Zervakis et al. 2001), but Gioia et al. (2005) could not find a significant difference in RAPD and minisatellite traits among the three varieties of the *P. eryngii* complex in Italy.

The host plant difference may not be important, but the difference of basidospore size was a question on the identification of Bai-Ling-Gu. And, if Bai-Ling-Gu were really *P. nebrodensis*, it should be compatible with *P. eryngii* of Europe.

We investigated the taxonomic position of Bai-Ling-Gu by mating, morphology, and sequencing of IGS1 and ITS1 sequences of rDNA. For taxonomic studies, comparison with the type is important. We studied the epitype and lectotype of *P. nebrodensis* designated by Venturella (2000). We could not obtain the type specimen of *P. eryngii* var. *tuoliensis* (Mou et al. 1987). Mao (2005) described that Bai-Ling-Gu was a cultured strain of *P. eryngii* var. *tuoliensis*. We could not find any types of *P. eryngii* and *P. eryngii* var. *ferulae*. Strain CBS 100.82 was studied by Zervakis and Balis (1996) and Zervakis et al. (2001), so we used it as a standard strain of *P. eryngii* var *eryngii*. The scientific name of CBS 282.32 is described as *P. fuscus* var. *ferulae*, and it is the synonym of *P. eryngii* var. *ferulae* (Saccardo 1887). We compared it with the Italian *Pleurotus* strains that grow on *Cachrys ferulacea* and *Ferula communis* (Gioia et al. 2005).

Materials and methods

Organisms

Strains used in this study are listed in Table 1. Some strains of Bai-Ling-Gu and A-Wei-Mo were obtained from Chinese research institutes. The strains of *P. eryngii* var. *ferulae* and *P. eryngii* var. *nebrodensis* of Italy were a gift from Prof. T. Gioia of Universita degli Studi Bari, Italy. These strains were maintained at the fungal culture collections of the Department of Biology and Plant Pathology, Università degli Studi di Bari, Italy. AFRL strains are stocked at the Asahimatsu Food Research Laboratory (Nagano, Japan). KBPO, KBPE, KBPB, KBPP, and PE strains are stocked at the Mushroom Laboratory, Forestry and Forest Products Research Institute (Ibaraki, Japan). All the cultures were maintained on potato dextrose agar (PDA) slants at the Asahimatsu Food Research Laboratory or the Mushroom Laboratory, Forestry and Forest Products Research Institute.

The epitype specimen of *Agaricus nebrodensis* was loaned from Herbier, Laboratoire de Cryptogamie, Muséum National d'Histoire Naturelle France (PC0088600).

Dual culture

Two strains were simultaneously seeded 2–3 cm apart on the same PDA plate and cultured for 3 weeks at 23°–24°C. The zone line formation was checked and recorded.

Mating

Monokaryotic strains were obtained by isolating germlings of the basidiospores. Incompatibility factors were determined by mating. Two monokaryotic strains were simultaneously seeded 2–3 cm apart on the same PDA plate and cultured for 7 weeks at 23°–24°C. Clamp connections were observed at the contact line and 3 cm apart from both sides from the contact line. The patterns of the colonies were also recorded as barrage, flat, and other. Fifteen monokaryotic strains were used for intrastrain crossing for a parent strain. Interstrain crossing was tested between eight monokaryotic strains, which consisted of two representatives for each tetrapolar mating type for a parent strain in principle. Di-mon mating was tested between a dikaryotic strain and eight monokaryotic strains from a parent strain.

Growth test of the mated mycelium

The mycelium 3 cm apart from the contact line was transferred to the center of a PDA plate and incubated at 23°–24°C for 10–13 days. Growth between day 3 and day 10 or 13 was recorded. When the growth of day 13 was reached the edge of the plate, the growth data between day 3 and day 10 were multiplied by 10/7 for the growth data of 10 days.

Table 1. Strains used in this study and the DDBJ/GenBank/EMBL accession number

Strain[a]	Species or variety		DDBJ/GenBank/EMBL accession no.		Host plant	Origin
	Labeled[b]	This study[c]	IGS1	ITS		
KBPO 1	*Pleurotus ostreatus*		AB234029			Commercial Strain of Child Woods Co. Ltd., Tsukuba, Japan
KBPO 2	*P. ostreatus*		AB234030			Commercial Strain of Taiwa Co., Nagano, Japan
KBPP1	*P. pulmonarius*		AB234031			Nagano Vegetable and Ornamental Crops Experimental Station, Nagano, Japan
CBS 100.82	*P. eryngii* var. *eryngii*	*P. eryngii* var. *eryngii*	AB234032		*Eryngium* sp.	Centraalbureau voor Schimmelcultures, Utreche, Netherlands
CBS 282.32	*P. fuscus* var. *ferulae*	*P. eryngii* var. *ferulae*	AB234033			Centraalbureau voor Schimmelcultures, Utreche, Netherlands
NBRC 32798	*P. eryngii*		AB234034			NITE Bioloical Resource Center, Chiba, Japan
KBPE 2	*P. eryngii*		AB234035			A fruit body in a market, Tsukuba, Japan
KBPE 4	*P. eryngii*		AB234036			A fruit body in a market, Tsukuba, Japan
KBPB 1	Bai-Ling-Gu	*P. eryngii* var. *tuoliensis*	AB234037			A fruit body in a market, Tsukuba, Japan
AFRL 6001	*P. eryngii*	*P. eryngii* var. *eryngii*				A fruit body in a market, Iida, Japan
AFRL 6011	Bai-Ling-Gu	*P. eryngii* var. *tuoliensis*	AB234038	AB286166, AB286170		Dr. Yamanaka of Kyoto Mycological Institute, Kyoto, Japan
AFRL 6012	Bai-Ling-Gu	*P. eryngii* var. *tuoliensis*	AB234039	AB286164, AB286168	*Ferula* sp.	Edible Fungi Institute, Shanghai Academy of Agricultural Science, Shanghai, China
AFRL 6013	Bai-Ling-Gu	*P. eryngii* var. *tuoliensis*	AB234040		*Ferula* sp.	Institute of Mycology, Sanming, Fujian, China
AFRL 6014	Bai-Ling-Gu	*P. eryngii* var. *tuoliensis*	AB234041	AB286163, AB286167	*Ferula* sp.	Cheng De Xian Ping Quan Xian Shi Yong Juen Zong Gong Si, Hebei, China
AFRL 6015	Bai-Ling-Gu	*P. eryngii* var. *tuoliensis*[d]	AB234042	AB286172, AB286174	*Ferula* sp.	TianShan 1 of the TianShanJun Y E, Xinjiang-Uygur Autonomous Region, China
AFRL 6016	Bai-Ling-Gu	*P. eryngii* var. *tuoliensis*	AB234043	AB286165, AB286169	*Ferula* sp.	TianShan 2 of the TianShanJun Y E, Xinjiang-Uygur Autonomous Region, China
AFRL 6017	Bai-Ling-Gu	*P. eryngii* var. *tuoliensis*	AB234044		*Ferula* sp.	TianShan 3 of the TianShanJun Y E, Xinjiang-Uygur Autonomous Region, China
AFRL 6021	A-Wei-Mo	*P. eryngii* var. *tuoliensis*[d]	AB234045, AB234047	AB286173	*Ferula* sp.	Institute of Mycology, Sanming, Fujian, China
AFRL 6022	A-Wei-Mo	*P. eryngii* var. *tuoliensis*[d]	AB234046	AB286171, AB286175	*Ferula* sp.	Cheng De Xian, Ping Quan Xian, Shi Yong Juen, Zong Gong Si, Hebei, China

Table 1. *Continued*

Strain[a]	Species or variety		DDBJ/GenBank/EMBL accession no.		Host plant	Origin
	Labeled[b]	This study[c]	IGS1	ITS		
DS 201	*P. eryngii* var. *nebrodensis*	*P. eryngii* var. *felulae/eryngii*	AB286121, AB286122	AB286161, AB286162	*Cachrys ferulacea*	Prof. Gioia of Università degli Studi di Bari, Italy; geographic origin: Senise (Potenza), Italy
DS 240	*P. eryngii* var. *ferulae*	*P. eryngii* var. *ferulae*	AB286123, AB286124	AB286159, AB286160	*Ferula communis*	Prof. Gioia of Università degli Studi di Bari, Italy; geographic origin: Gravina (Bari), Italy
DS 247	*P. eryngii* var. *ferulae*	*P. eryngii* var. *ferulae*	AB286125, AB286126	AB286157, AB286158	*Ferula communis*	Prof. Gioia of Università degli Studi di Bari, Italy; geographic origin: Altamura (Bari), Italy
DS 260	*P. eryngii* var. *nebrodensis*	*P. eryngii* var. *ferulae*	AB286127, AB286128	AB286155, AB286156	*Ferula communis*	Prof. Gioia of Università degli Studi di Bari, Italy; geographic origin: Altamura (Bari), Italy
DS 264	*P. eryngii* var. *ferulae*	*P. eryngii* var. *ferulae*	AB286129, AB286130	AB286154	*Ferula communis*	Prof. Gioia of Università degli Studi di Bari, Italy; geographic origin: Incoronata (Foggia), Italy
DS 388	*P. eryngii* var. *ferulae*	*P. eryngii* var. *ferulae*	AB286131, AB286132	AB286152, AB286153	*Cachrys ferulacea*	Prof. Gioia of Università degli Studi di Bari, Italy; geographic origin: Putignano (Bari), Italy
DS 391	*P. eryngii* var. *nebrodensis*	*P. eryngii* var. *nebrodensis*	AB286133, AB286134	AB286150, AB286151	*Cachrys ferulacea*	Prof. Gioia of Università degli Studi di Bari, Italy; geographic origin: Parco delle Madonie (Palermo) Sicily, Italy
DS 393	*P. eryngii* var. *nebrodensis*	*P. eryngii* var. *nebrodensis*	AB286135	AB286148, AB286149	*Cachrys ferulacea*	Prof. Gioia of Università degli Studi di Bari, Italy; geographic origin: Sicily, Italy
DS 504	*P. eryngii* var. *nebrodensis*	*P. eryngii* var. *nebrodensis*	AB286136, AB286137	AB286145, AB286147	*Cachrys ferulacea*	Prof. Gioia of Università degli Studi di Bari, Italy; geographic origin: Parco delle Madonie (Palermo) Sicily, Italy
DS 506	*P. eryngii* var. *nebrodensis*	*P. eryngii* var. *nebrodensis*	AB286138, AB286139	AB286144, AB286146	*Cachrys ferulacea*	Prof. Gioia of Università degli Studi di Bari, Italy; geographic origin: Parco delle Madonie (Palermo) Sicily, Italy
PE_deutsch	*P. eryngii*		AB286140, AB286141			Germany
PE_czech	*P. eryngii*		AB286142, AB286143			Czech
PC 0088600	*Agaricus nebrodensis*	*P. eryngii* var. *nebrodensis*	AB272089[e], AB272090[e]	AB272091[e], AB272092[e]	*Cachrys ferulacea*	Herbier, Laboratoire de Cryptogamie, Muséum National d'Histoire Naturelle, France; geographic origin: Sicily, Italy

ITS, internal transcribed spacer region; IGS, intergenic spacer

[a] KBPO, KBPE, KBPB, KBPP, and PE strains are stocked at the Mushroom Laboratory, Forestry and Forest Products Research Institute, 1 Matsunosato, Tsukuba, Ibaraki 305-8687, Japan; AFRL strains are stocked at Asahimatsu Food Research Laboratory, 1008 Dashina, Iida, Nagano 399-2561, Japan; DS strains are preserved at the fungal culture collection at the Department of Biology and Plant Pathology, Università degli Studi di Bari, Italy; CBS (Centraalbureau voor Schimmelcultures, the Netherlands); NBRC (NITE Biological Resource Center, Department of Biotechnology, National Institute of Technology and Evaluation, Japan)

[b] Species or variety following the distributor

[c] Species or variety estimated in this study

[d] Corresponding to group 3 by Zhang et al. (2006)

[e] Partial sequence

Cultivation of fruit bodies

The parent strains and some of the mated dikaryotic strains were tested for fruiting. The substrate was composed of cotton hull:corncob:wheat bran:corn bran:$CaCO_3$ at 39:35:20:5:1 (dry weight ratio) and 65% moisture. About 550g substrate was packed in an 850-ml plastic bottle. The bottle was sterilized at 121°C for 60min following making a hole of 10mm diameter in the center. After cooling to 20°C, the mycelium with agar medium that had been prepared in the growth test was spawned. The bottle was incubated at 22°–24°C for 49 days. After scraping off a part of the surface of the mycelial block, the bottle was kept at 5°–7°C for 14 days for low-temperature treatment, and was moved to a cropping room of 15°–17°C with fluorescent light of 100–200lx. The low-temperature treatment was omitted, excepting Bai-Ling-Gu, for other than AFRL 6015. Fruit bodies harvested within 49 days in the cropping room were recorded.

DNA extraction and PCR amplification

Whole-genome DNA was extracted from fresh mycelia and from the gill fractions of the type specimen according to the method of Lee and Taylor (1990). The IGS1 region, including 96bp of the 3′-end of the 28S rDNA, IGS1, and 107bp of the 5′-end of the 5S rDNA were amplified by polymerase chain reaction (PCR) using primers P-1 (5′-TTGCAGACGACTTGAATGG-3′) (Hsiau 1996) and 5s_rdn-1 (5′-TAGGATTCCCGCGTGGTCCCCCA-3′). Primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) of White et al. (1990) were used for amplification of ITS region (3′-end of 18S rDNA, ITS1, 5.8S rDNA, ITS2, and 5′-end of 28S rDNA). To recover species-specific DNA segments of ITS1 and IGS1 of *P. nebrodensis* epitype DNA, primer sets ITSp (Pleurotus_ITS1p_f1:5′-cttcac tagtctttcaaccacctgtgaac-3′, Pleurotus_ITS1p_r2: 5′-tgaaagtt gtattatggtttataggcac-3′) and IGS1p (Pleurotus_IGS1p_f1: 5′-caataaggtcatcagcaatgaaactgac-3′, Pleurotus_IGS1p_r2: 5′-gggttcaacatcacaaaggggggaatatag-3′) were used, respectively. PCR reactions were carried out using TaKaRa LA Taq (Takara Bio, Shiga, Japan) according to the manufacturer's specifications. The PCR amplicons were electrophoresed in 1.5% agarose gels in TBE buffer. The desired band was visualized under a long wavelength ultraviolet light and cut from the gel. The DNAs were eluted from the gel using Ultrafree-MC 0.45-μm filter units (Millipore, Bedford, MA, USA) and cloned into pGEM-T-Easy vector (Promega, Madison, WI, USA) or pCR2.1 vector (Invitrogen, Carlsbad, CA, USA) by T/A cloning.

DNA sequencing and data analysis

The plasmids containing the target inserts were sequenced in both directions using the M13 universal forward and reverse primers or the primers P-1 and 5S_rdn-1 labeled with IRDye™ 700 or 800 (Li-Cor, Lincoln, NE, USA). Sequences were determined on at least three plasmid clones prepared in each strain. Sequence reactions were conducted with Thermo Sequenase Cycle Sequencing Kit (USB, Cleveland, OH, USA) following the manufacturer's instructions and run on an a NEN Global IR2 DNA Sequencer System (Li-Cor). DNA sequences were aligned using multiple sequence alignment with hierarchical clustering (Corpet 1988) on the INRA URL (http://bioinfo.genopole-toulouse.prd.fr/multalin/multalin.html) or Clustal W program on the DDBJ URL (http://clustalw.ddbj.nig.ac.jp/top-e.html) with default setting. Phylogenetic analyses were also performed by using the Clustal W program of the DDBJ URL with neighbor-joining algorithm and the distance option of Kimura's correction. Bootstrap neighbor-joining analyses were carried out with 1000 replicates. The phylogenetic trees were visualized and printed with Njplot (Perrière and Gouy 1996).

Sequences determined in this study were deposited and are available in the DDBJ database under the accession numbers shown in Table 1. The strains and accession numbers of sequences downloaded from DDBJ/GenBank/EMBL for analyses were as follows: *P. pulmonarius* CCRC36230 (AY265841), TMI30385 (AY368669), and HMAS76672 (AY696299); *P. ostreatus* CCRC36249 (AY265841), S474 (AY540322), OE-43 (AY265841), and wc534 (AF079583); *P. eryngii* ATCC36047 (AY368657) and *P. eryngii* var. *ferulae* ACCC50656 (AY463033); Chinese *Pleurotus* cultivar No. 4 Bailing (AY720935) and Chinese *P. nebrodensis* ACCC50869 (AY463034).

Results

Dual cultures

Table 2 shows the results of dual cultures among the several strains in Table 1. AFRL 6011–6014 and AFRL 6016–6017 did not form a zone line with these strains but formed zone lines with the other strains. These six strains must be closely related. AFRL 6015 and AFRL 6021–6022 also did not form a zone line between these strains, but formed zone lines with the other strains. Although AFRL 6015 was labeled Bai-Ling-Gu and AFRL 6021 and 6022 were labeled A-Wei-Mo, they could be closely related.

Cultivation of fruit bodies

All strains listed in Table 1 except KBP and PE strains were cultivated. AFRL 6011–6014 and AFRL 6016–6017 needed low-temperature treatment to fruit, and the fruit bodies were almost the same as those of AFRL 6016 (Fig. 1A). The other strains fruited without the low-temperature treatment. The fruit bodies of AFRL 6021 and 6022 were almost the same as those of AFRL 6015 (Fig. 1B). These three strains should be closely related, as already stated. The rest of the strains gave fruit bodies with an individual shape (Fig. 1C–N).

Table 2. Dual culture of several strains of *Pleurotus eryngii* sensu lato

Strain[a]	NBRC 32798	CBS 100.82	CBS 282.32	AFRL 6001	AFRL 6011	AFRL 6012	AFRL 6013	AFRL 6014	AFRL 6015	AFRL 6016	AFRL 6017	AFRL 6021	AFRL 6022
NBRC 32798	–	+	+	+	+	+	+	+	+	+	+	+	+
CBS 100.82	+	–	+	+	+	+	+	+	+	+	+	+	+
CBS 282.32	+	+	–	+	+	+	+	+	+	+	+	+	+
AFRL 6001	+	+	+	–	+	+	+	+	+	+	+	+	+
AFRL 6011	+	+	+	+	–	–	–	–	+	–	–	+	+
AFRL 6012	+	+	+	+	–	–	–	–	+	–	–	+	+
AFRL 6013	+	+	+	+	–	–	–	–	+	–	–	+	+
AFRL 6014	+	+	+	+	–	–	–	–	+	–	–	+	+
AFRL 6015	+	+	+	+	+	+	+	+	-	+	+	–	–
AFRL 6016	+	+	+	+	–	–	–	–	+	–	–	+	+
AFRL 6017	+	+	+	+	–	–	–	–	+	–	–	+	+
AFRL 6021	+	+	+	+	+	+	+	+	–	+	+	–	–
AFRL 6022	+	+	+	+	+	+	+	+	–	+	+	–	–

+, formed zone; –, did not form zone line
[a] See Table 1

Table 3 summarizes the shape of the cultivated fruit bodies. AFRL 6016 has a large white cap and a very short stem, whereas AFRL 6015 has a white cap and a thick and long stem. Both strains were designated as Bai-Ling-Gu by the TianShanJun Y E, Xinjiang-Uygur Autonomous Region, China. DS 201, 260, 391, 393, 504, and 506 were designated as *P. eryngii* var. *nebrodensis*. DS 260 had a charcoal-colored cap, as did DS 240, 247, 264, and 388, which were designated as *P. eryngii* var. *ferulae*. The other strains had whitish big caps with cream lamellae, except DS 201; DS 201 had small caps and long stems with apricot lamellae.

Table 4 shows the size of the basidiospores of several strains of *P. eryngii* sensu lato. The basidiospores of the epitype of *P. nebrodensis*, DS 391, and DS 393 were larger than those of the other strains. DS 201 and DS 260 differed in shape of fruit body, color of lamella, and size of basidiospores from DS 391 and DS 393. The data of the latter strains did not contradict those of the epitype of *P. nebrodensis* and several published reports (Venturella 2000; Zervakis et al. 2001).

Mon-mon mating

Monokaryotic strains were obtained from some of the cultivated fruit bodies and the mating types were determined. CBS 100.82, CBS 282.32, AFRL 6011, AFRL 6016, and AFRL 6021 exhibited tetrapolarity. Eight monokaryotic strains were selected from each parent dikaryon. Two monokaryotic strains of the same mating type were basically chosen. In all the 20 (5 strains, tetrapolarity) mating types, only 1 monokaryotic strain was obtained in the 2 mating types. In that case, it was substituted by a strain of another mating type. Table 5 shows the mating ratio and the growth rate of the mated mycelia. Between AFRL 6016 and AFRL 6021, the mating ratio was 100%. Thus, these 2 strains must be the same species. Between CBS 100.82 and CBS 282.32, the mating ratio was 98%; these 2 strains should be the same species. The mating ratio of AFRL 6016 with CBS 100.82 and CBS 282.32 was 65% and 82%, respectively. The many mated mycelia showed stunted growth, but several mycelia grew as fast as the mated mycelia between CBS 100.82 and CBS 282.32. All the strains could be the same species, but they might be divided into a couple of varieties.

Di-mon mating

Each of the eight monokaryotic strains from AFRL 6016 (Bai-Ling-Gu), CBS 282.32 (*P. eryngii* var *ferulae*), and CBS 100.82 (*P. eryngii* var. *eryngii*) were mated with several dikaryons (Table 6). In the di-mon mating of Bai-Ling-Gu and A-Wei-Mo with monokaryotic strains of AFRL 6016 (Bai-Ling-Gu), almost all were mated, and the growth rates of the mated mycelia were nearly the same as those of the original monokaryons. In that with CBS 282.32 (*P. eryngii* var. *ferulae*), the mating ratio was 79% (30/38), and the growth was slow in more than half the mated mycelia. In that with CBS 100.82 (*P. eryngii* var. *eryngii*), only 1 of 40 combinations was mated.

In the di-mon mating of *P. eryngii* var. *ferulae* with monokaryotic strains of AFRL 6016 (Bai-Ling-Gu), almost all were mated, and the growth rates of the mated mycelia were about half of those of the original monokaryons. In the di-mon mating of *P. eryngii* var. *ferulae* with monokaryotic strains of CBS 282.32 (*P. eryngii* var. *ferulae*), the mating ratio were 75% (30/40). In that with CBS 100.82 (*P. eryngii* var. *eryngii*), no combination was mated.

In the di-mon mating of *P. eryngii* var. *eryngii* with monokaryotic strains of AFRL 6016 (Bai-Ling-Gu), the mating ratio was 13% (2/16). In that with monokaryotic strains of CBS 282.32 (*P. eryngii* var. *ferulae*), the mating ratio was 100% (14/14), and the growth rates of mated mycelia were nearly the same or better than the original monokaryon. In that with CBS 100.82 (*P. eryngii* var. *eryngii*), the mating ratio was 100% (16/16) and the growth rates of the mated mycelia were good.

In the di-mon mating of *P. eryngii* var. *nebrodensis* of Italy, DS 391, DS 393, DS 504, and DS 506 showed nearly

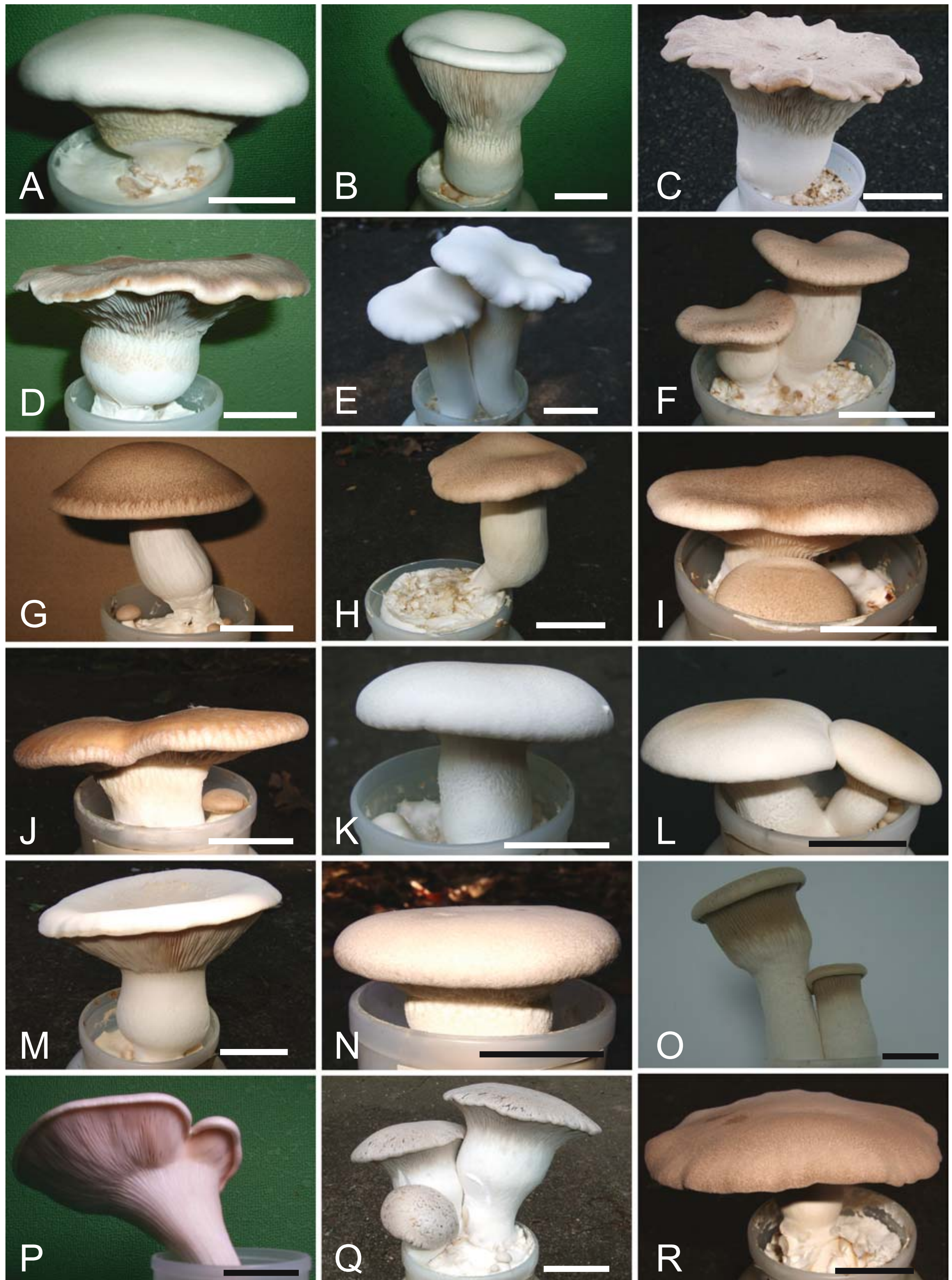

Fig. 1. Cultivated fruit bodies. **A** AFRL 6016. **B** AFRL 6015. **C** CBS 100.82. **D** CBS 282.32. **E** DS 201. **F** DS 240. **G** DS 247. **H** DS 260. **I** DS 264. **J** DS 388. **K** DS 391. **L** DS 393. **M** DS 504. **N** DS 506. **O** A hybrid between AFRL 6011 and AFRL 6001. **P** Another hybrid between AFRL 6011 and AFRL 6001. **Q** A F_2 hybrid between AFRL 6011 and AFRL 6001. **R** A hybrid between CBS 100.82-22 and DS 391. *Bars* 30 mm

Table 3. Cultivated fruit bodies of several *Pleurotus* strains

Strain[a]	Cap	Lamella	Stem
CBS 100.82	100–140 mm, flat, pale brown	Decurrent, pale yellow brown	D 50 × H 42 mm, central, white
CBS 282.32	100–130 mm, flat, pale brown	Adnate-decurrent, pale yellow brown	D 51 × H 34 mm, central, white
AFRL 6015	70–100 mm, flat or centrally depressed, white	Decurrent, dull white	D 50 × H 50 mm, central, white
AFRL 6016	100–150 mm, flat convex, white	Decurrent, cream	D 20 × H 15 mm, excentric, white
DS 201	55–80 mm, flat, white	Decurrent, pale apricot	D 26 × H 81 mm, central, white, upper part tinged yellow
DS 240	41–52 mm, flat, pale brown	Decurrent, pale brown	D 26 × H 48 mm, central, white
DS 247	84–98 mm, convex, brown	Decurrent, pale yellow brown	D 41 × H 95 mm, central, white
DS 260	51–53 mm, flat, pale brown	Decurrent, pale yellow brown	D 25 × H 55 mm, central, white
DS 264	62–86 mm, flat, brown	Decurrent, pale yellow brown	D 28 × H 46 mm, central, white
DS 388	52–56 mm, flat, pale brown	Decurrent, pale yellow brown	D 30 × H 38 mm, central, white
DS 391	65–108 mm, flat convex, white, center tinged pale brown	Decurrent, cream	D 32 × H 38 mm, central, white
DS 393	56–93 mm, flat convex, white, center tinged pale brown	Decurrent, cream	D 32 × H 42 mm, central, white
DS 504	100–105 mm, flat-convex or centrally depressed, cream, center tinged pale orange	Decurrent, cream	D 41 × H 48 mm, central, white
DS 506	58–60 mm, flat convex, pale yellow brown	Decurrent, cream	D 32 × H 29 mm, central, white

D, diameter; H, height
[a] See Table 1

Table 4. Size of basidiospores of the strains of *Pleurotus eryngii* sensu lato

Strain or source[a]	Species or variety[b]	Length (μm)	Width (μm)
Zervakis et al. 2001	*P. eringii* var. *eringii*	9.1–13.5	4.8–6.7
Zervakis et al. 2001	*P. eryngii* var. *ferulae*	9.6–13.8	4.7–6.9
Zervakis et al. 2001	*P. nebrodensis*	13.2–17.4	5.5–8.2
Mao 2005	Bai-Ling-Gu, *P. nebrodensis*	10.8–14	4.8–6
Mao 2005	A-Wei-Mo, *P. ferulae*	12–14	5–7
Epitype	*Agaricus nebrodensis*	10–15	5.5–7.5
AFRL 6001	*P. eryngii*	9–11.5	4–5
AFRL 6015	Bai-Ling-Gu	10–13.5	4.5–6
AFRL 6016	Bai-Ling-Gu	10–14	4.5–6
DS 201	*P. eryngii* var. *nebrodensis*	9–12	4–6
DS 260	*P. eryngii* var. *nebrodensis*	9–13	4.5–6
DS 264	*P. eryngii* var. *ferulae*	10–12	4.5–5.6
DS 391	*P. eryngii* var. *nebrodensis*	11–16	5–8
DS 393	*P. eryngii* var. *nebrodensis*	11–16	5–8

[a] The first five data are from the papers cited, and the rest are our observations (see Table 1)
[b] Following the name of the distributor (labeled)

Table 5. Mating rate and average growth rate of the hybrids by the mon-mon mating of *Pleurotus eryngii* sensu lato

Mon-mon mating		Number		Mating	Growth (mm/10 day)
Original dikaryon[a]	Original dikaryon[a]	Tested	Mated	(%)	(average ± SD)
AFRL 6016	AFRL 6021	128	128	100	21 ± 8
AFRL 6016	CBS 100.82	128	83	65	13 ± 7
AFRL 6016	CBS 282.32	128	105	82	14 ± 6
CBS 100.82	CBS 282.32	128	125	98	29 ± 12

[a] See Table 1

the same pattern: they mated with every monokaryon of CBS 100.82 (*P. eryngii* var. *eryngii*) but hardly mated with the monokaryons of AFRL 6016 (Bai-Ling-Gu) and CBS 282.32 (*P. eryngii* var. *ferulae*), The di-mon mating pattern of DS 260 looked like that of *P. eryngii* var. *ferulae.* The di-mon mating pattern of DS 201 was peculiar. The mating ratios of DS 201 with AFRL 6016 (Bai-Ling-Gu), CBS 282.32 (*P. eryngii* var. *ferulae*), and CBS 100.82 (*P. eryngii* var. *eryngii*) were 63% (5/8), 100% (8/8), and 63% (5/8), respectively.

Table 6. Growth rate of the mated hyphae in the di-mon mating of *Pleurotus eryngii* sensu lato

Fungal name/strain[a]					Bai-Ling-Gu			A-Wei-Mo/ Bai-Ling-Gu		*Pleurotus eryngii*		*P. eryngii* var. *ferulae*					*P. eryngii* var. *nebrodensis*					
Original dikaryotic strain	Monokaryotic strain number	Incompatibility factor		Growth rate[b]	AFRL	AFRL	AFRL	AFRL	AFRL	CBS	AFRL	CBS	DS	DS	DS	DS	DS	DS	DS	DS	DS	DS
		A	B		6016	6011	6012	6021	6015	100.82	6001	282.32	240	264	247	388	201	260	391	393	504	506
Bai-Ling-Gu AFRL 6016	2	11	16	27[b]	11[b]	18	15	26	21	–	–	15	15	9	7	–	17	11	–	–	–	–
	24	11	16	32	14	26	19	15	33	–	–	14	14	14	9	9	–	13	–	–	–	–
	36	11	17	34	33	35	35	33	35	–	5	15	24	15	11	8	14	12	–	–	–	–
	100	11	17	33	34	32	35	34	21	–	–	15	23	14	10	7	–	–	–	–	–	–
	50	12	16	33	28	18	23	13	21	–	–	15	19	16	15	7	16	14	–	–	–	–
	76	12	16	29	29	26	18	23	21	–	–	12	18	14	8	10	12	15	7	–	–	33
	65	12	17	29	26	16	21	36	30	–	40	12	18	–	12	8	14	–	9	–	–	–
	83	12	17	23	–	–	20	30	18	–	–	18	–	–	17	–	–	–	–	–	–	–
P. fuscus var. *ferulae* CBS 282.32	20	71	76	30	–	15	17	31	–	34	26	–	16	–	18	17	23	14	–	–	–	–
	87	71	76	26	–	13	16	10	23	50	42	–	40	16	57	21	26	19	–	–	–	–
	62	71	76	20	–	n.d.	–	9	n.d.	n.d.	n.d.	18	16	13	29	21	24	23	–	–	–	–
	12	71	77	27	21	11	25	23	–	18	42	12	–	20	–	–	31	18	–	–	–	–
	13	72	76	25	6	17	13	8	5	35	20	23	17	10	–	–	25	19	–	–	–	–
	17	72	76	19	–	–	26	6	6	19	50	24	20	8	20	17	28	15	–	–	–	–
	70	72	77	27	47	14	20	32	24	16	18		18	12	31	32	20	12	–	–	–	–
	73	72	77	26	19	14	18	18	19	21	15	–	5	11	18	16	27	12	–	–	–	–
P. eryngii var. *eryngii* CBS 100.82	22	51	56	32	–	–	–	–	–	50	53	–	–	–	–	–	37	–	21	29	8	24
	66	51	58	37	–	–	–	–	–	37	48	–	–	–	–	–	22	–	5	6	6	5
	45	51	57	39	–	–	–	–	–	42	48	–	–	–	–	–	–	–	4	12	10	5
	121	51	57	32	–	–	–	–	–	43	40	–	–	–	–	–	43	–	5	8	10	8
	109	52	56	39	–	–	–	–	–	35	52	–	–	–	–	–	29	–	5	7	8	7
	123	52	56	39	–	–	–	–	12	58	70	–	–	–	–	–	–	–	6	24	6	21
	69	52	57	28	–	–	–	–	–	45	37	–	–	–	–	–	19	–	22	25	7	14
	17	52	57	29	–	–	–	–	–	26	46	–	–	–	–	–	–	–	28	33	5	9

–, did not mate; n.d., no data

[a] Horizontal rows are fungal names and strain numbers of dikaryons; vertical rows are fungal names and strain numbers of the original dikaryons, and the strain numbers of the derived monokaryons; the variety names followed the distributor (see Table 1)

[b] Growth rate (mm/10 days)

Cultivation of the hybrid mycelium

For the cultivation test, mon-mon mating between AFRL 6001 and AFRL 6011 was used. The number of mated mycelia was 232 of 520 crossings, and 43 strains were selected by the growth test. Within 98 days of cultivation, 32 strains formed fruit bodies (Fig. 1O,P). The shapes of the fruit bodies were intermediates of the two parental strains. One strain formed a white fruit body (Fig. 1P), but the other strains formed fruit bodies with pale brown caps and pale brown gills (Fig. 1O). Many monokaryotic mycelia were obtained by isolating germlings of the basidiospores of the F_1 fruit bodies. The monokaryotic mycelia were crossed, and mated mycelia were cultivated. Many F_2 mycelia were fruited (Fig. 1Q). Some di-mon hybrids between *P. eryngii* var. *nebrodensis* and *P. eryngii* var. *eryngii* fruited (Fig. 1R).

Molecular analysis of the type specimen of *Agaricus* nebrodensis Inzenga (= *Pleurotus nebrodensis* (Inzenga) Quél.)

To utilize it as an authentic DNA reference of *P. nebrodensis*, DNA recovery from the epitype specimen of *A. nebrodensis* Inzenga (PC0088600) was carried out. The recovered DNA samples were too small to use as a template DNA for PCR amplification of the complete sequence of the IGS1 or ITS region. Therefore, partial segments with species-specific and polymorphic nucleotide sequences of 235 bp and 138 bp of the IGS1 and ITS1, respectively, were amplified and sequenced. Molecular analysis revealed that only Sicilian *P. eryngii* var. *nebrodensis* strains in all tested strains possessed the nucleotide sequences identical to those of the segments of the epitype specimen in the corresponding regions of IGS1 and ITS1, and the strains of *P. eryngii* var. *nebrodensis* of the Italian Peninsula and Bai-Ling-Gu had the other types of nucleotide sequences. Thus, it was found to be not correct to use *P. eryngii* var. *nebrodensis* or *P. nebrodensis* as the scientific name of the *Pleurotus* strains of Italy and China.

Phylogenetic analysis based on ITS1 sequence

To clarify the taxonomic position of Chinese *Pleurotus* “Bai-Ling-Gu,” molecular phylogenetic analysis of Bai-Ling-Gu and the related strains was performed using ITS and IGS1 DNA sequences. As the reference strains, some culture collections, *P. eryngii* var. *nebrodensis*, and *P. eryngii* var. *ferulae* strains from Italy were used. Some data from the DDBJ database were also inserted.

The ITS region that consists of ITS1, 5.8S rDNA, and ITS2 ranged from 633 to 635 bp in the Bai-Ling-Gu and labeled A-Wei-Mo strains. The size variation was caused by different nucleotide numbers of T simple-repeated sequence in the ITS2 region. In contrast, those of the DS strains were consistently 633 bp. Comparison within the Bai-Ling-Gu, Sicilian *P. eryngii* var. *nebrodensis* strains, and Italian *P. eryngii* var. *ferulae* strains on ITS nucleotide sequences revealed that these mushroom groups were clearly distinguished from each other based on base substitution or insertion/deletion polymorphism of 15 base positions. Figure 2 shows the phylogenetic tree inferred by ITS1 nucleotide sequence analysis of the relevant *Pleurotus* spp. including *P. ostreatus* and *P. pulmonarius* as outgroups. The ITS1 sequence data of *P. osteatus*, *P. pulmonarius*, and some strains of *P. eryngii* sensu lato were derived from DDBJ/Genbank/EMBL primary data. The strains of *P. eryngii* sensu lato were apparently divided into three clades. The clade of Bai-Ling-Gu consisted of Bai-Ling-Gu strains (AFRL 6011, 6012, 6014, 6015, 6016, and No. 4 Bailing) and labeled A-Wei-Mo strains (AFRL 6021 and 6022). The scientific name of No. 4 Bailing was described as *P. nebrodensis* (AY720935). The clade of *P. eryngii* var. *nebrodensis* (Sicily) consisted of DS 391, 393, 504, and 506. All these strains originated in the Sicily Island. The clade of the *P. eryngii* var. *eryngii-ferulae* complex consisted of *P. eryngii* of Czechoslovakia (ATCC 36047), which had grown on *Eryngium* sp. (Zervakis et al. 2001), Italian *P. eryngii* var. *ferulae* strains (DS 240, 247, 264, and 388), and labeled *P. eryngii* var. *nebrodensis* strains originated in the Italian Peninsula (DS 201 and 260). The bootstrap supported 96.8% and 96.0% for the clades of Bai-Ling-Gu and that of *P. eryngii* var. *nebrodensis* (Sicily), respectively.

Phylogenetic analysis based on IGS1 sequences

To confirm the foregoing results, the IGS1 sequences of three mushroom groups were additionally analyzed. The IGS1s of the Bai-Ling-Gu or labeled A-Wei-Mo were divided into two groups by length. One was a major type of 645 bp that was found in all given strains of Bai-Ling-Gu and labeled A-Wei-Mo, except Bai-Ling-Gu strain AFRL 6015 and the other was a minority of 642 bp in Bai-Ling-Gu strain AFRL 6015 and labeled A-Wei-Mo strain AFRL6021. In contrast, the IGS1s of Sicilian *P. eryngii* var. *nebrodensis* and *P. eryngii* var. *eryngii-ferulae* complex strains were 645 bp, and within a range from 644 to 646 bp, respectively, in length. Alignment analysis of the nucleotide sequence of the IGS1s demonstrated once more that the three mushroom groups differed from one another in nucleotide substitution or insertion/deletion polymorphism of 13 base positions. Figure 3 shows a phylogenetic tree based on the IGS1 nucleotide sequence analysis of the relevant *Pleurotus* spp. As well as that of the ITS1, Bai-Ling-Gu, and labeled A-Wei-Mo, Sicilian *P. eryngii* var. *nebrodensis* and *P. eryngii* var. *eryngii-ferulae* complex were found to be classified evidently into three independent clades, which were supported by a bootstrap value ranging from 61.1% to 97.2%.

The clade of Bai-Ling-Gu consisted of Bai-Ling-Gu (AFRL 6011, 6013, 6014, 6015, 6016, 6017, and KBPB 1), labeled A-Wei-Mo (AFRL 6021 and 6022), and Chinese labeled *P. nebrodensis* (ACCC 50869). The clade of *P. eryngii* var. *nebrodensis* (Sicily) consisted of DS 391, 393, 504, and 506. The clade of *P. eryngii* var. *eryngii-ferulae* complex consisted of *P. eryngii* strain (CBS 100.82, NRBC 32798, KBPE 2, 4, PE_deutsch, and PE_czech), *P. eryngii*

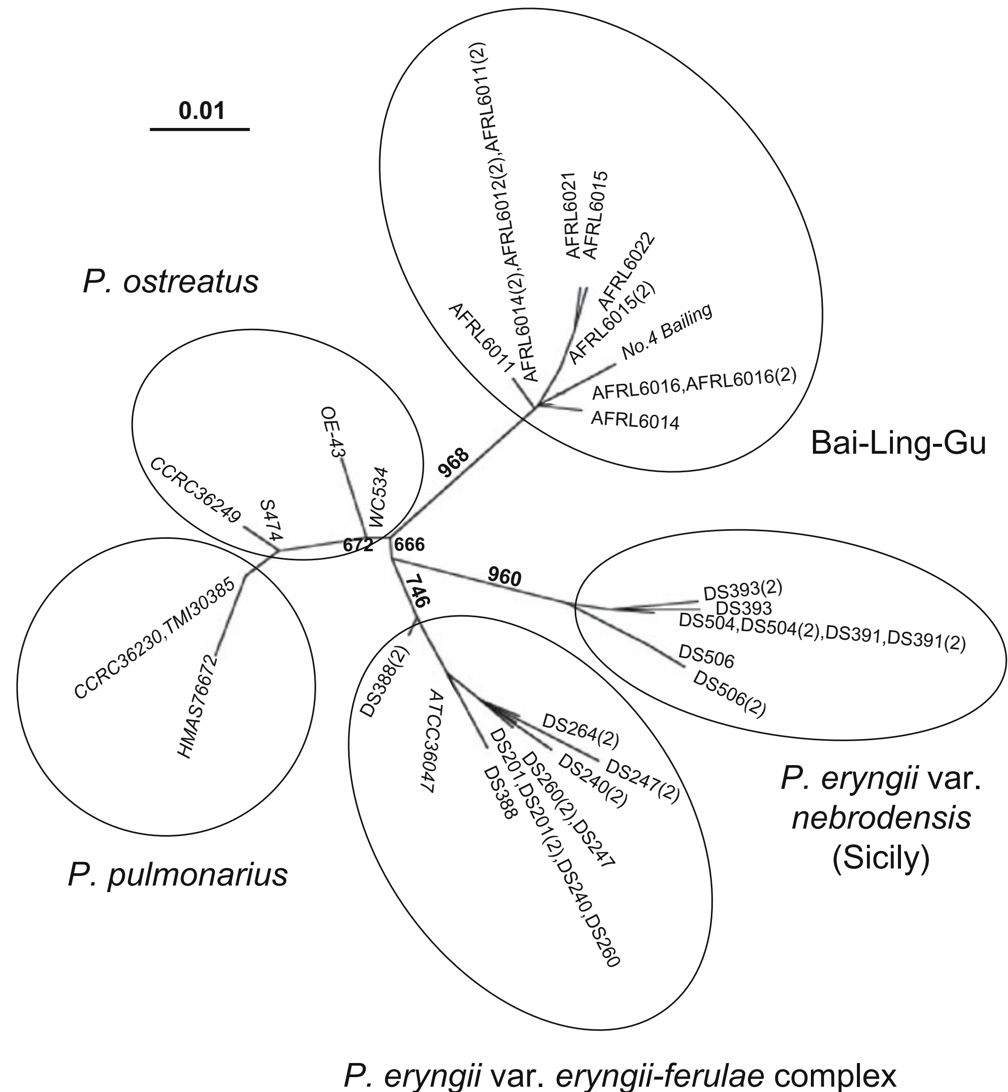

Fig. 2. Phylogenetic tree inferred by analysis of rDNA internal transcribed spacer (ITS)1 sequences of *Pleurotus* spp. *Numbers close to branches* indicate bootstrap support in 1000 replications. ITS1 sequences of *P. pulmonarius* CCRC36230 (AY265841), TMI30385 (AY368669), and HMAS76672 (AY696299), *P. ostreatus* CCRC36249 (AY265841), S474 (AY540322), OE-43 (AY265841), and WC534 (AF079583), and Chinese *Pleurotus* cultivars no. 4 Bai-Ling (AY720935) and *P. eryngii* ATCC36047 (AY368657) were downloaded from DDBJ/GenBank/EMBL. *Scale bar* indicates 0.01 substitutions/site

var. *ferulae* strains (CBS 282.32, DS 240, 247, 264, 388, and ACCC 50656), and labeled *P. nebrodensis* strains originated from the Italian Peninsula (DS 201 and 260). The host plant of ACCC 50656 was growing on *Ferula sinkiangensis* (Zhang et al. 2006), and the strain was a real A-Wei-Mo.

Discussion

In taxonomy, it is important to compare a questioned strain with type cultures. There are no type cultures of *P. eryngii*, *P. eryngii* var. *ferulae*, and *P. eryngii* var. *nebrodensis*, so we used some strains from culture collections. CBS 100.82 was used by Zervakis and Balis (1996) and Zervakis et al. (2001) and gave the standard data for the strain of *Pleurotus eryngii* var. *eryngii*. The host plant from which the strain was isolated was *Eryngium* sp. in Slovakia. CBS 282.32 was named *Pleurotus fuscus* var. *ferulae* Lanzi. *P. fuscus* var. *ferulae* is the synonym of *P. eryngii* var. *ferulae* (Lanzi) Saccardo, (Saccardo 1887).

Six strains named Bai-Ling-Gu did not form zone lines with each other in the dual culture and formed nearly the same fruit bodies. Two of them had the same mating type (data not shown), so they may be the same strain or sister strains. Tan et al. (2006) suggested that five of six Bai-Ling-Gu strains commercially cultivated in China were originally isolated from the same source, as indicated by their morphology and DNA fingerprinting. Jia and Qin (2006) reported that four major strains were used in the cultivation of Bai-Ling-Gu and that ACCC 50869 was the most common. All the Bai-Ling-Gu strains, except AFRL 6515, listed in Table 2 could be closely related to ACCC 50869.

One strain labeled Bai-Ling-Gu and two strains labeled A-Wei-Mo did not form a zone line to each other in the dual culture, formed similar fruit bodies, and had the same mating factors (data not shown); they also could be the same strain or sister strains. The host plants of both strains were *Ferula* sp. (Huang 1998; Mao 2000), and the naming might be confused. Zhang et al. (2006) reported the detailed analysis of one *P. eryngii* var. *ferula* strain (ACCC 50656). The host plant was *Ferula sinkiangensis*. The ITS sequence

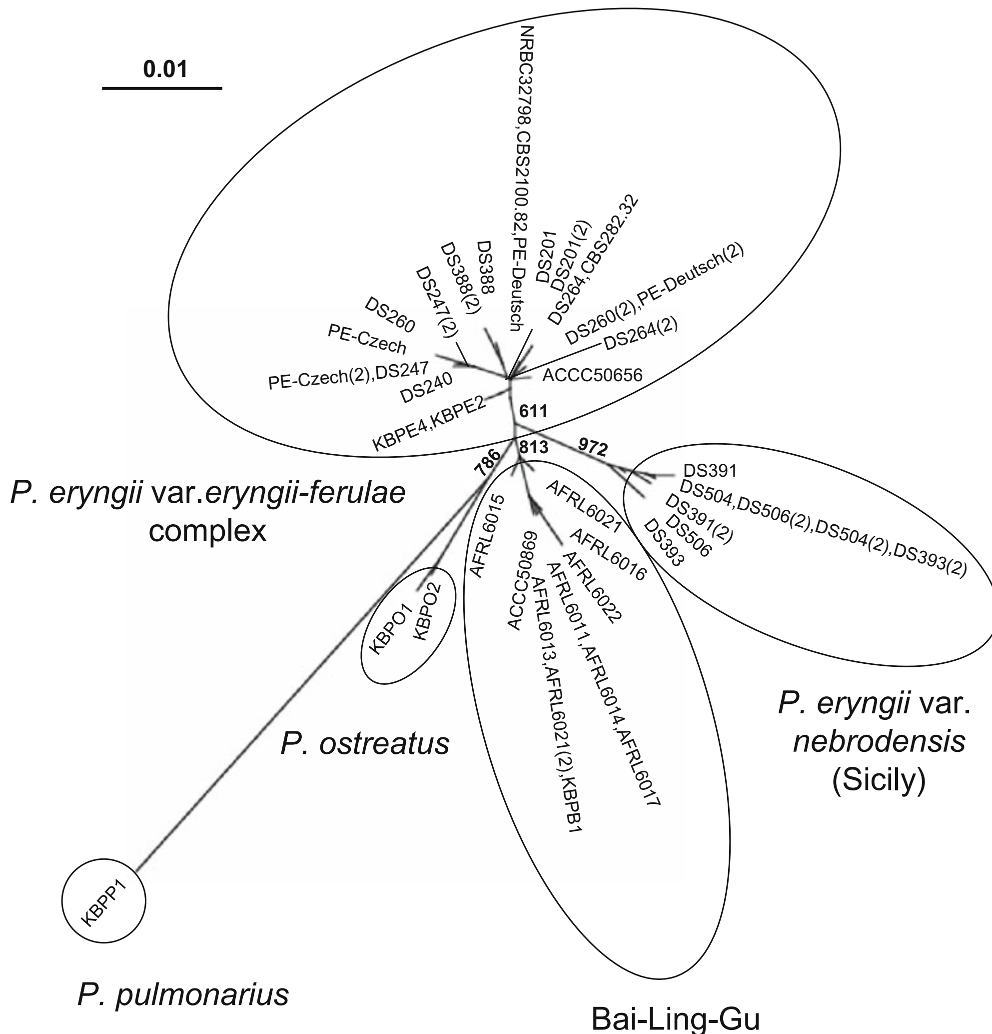

Fig. 3. Phylogenetic tree inferred by analysis of rDNA intergenic spacer (IGS)1 sequences of *Pleurotus* spp. *Numbers close to branches* show bootstrap support in 1000 replications. The IGS1 sequences of *P. eryngii* var. *ferulae* ACCC50656 (AY463033) and Chinese *Pleurotus* cultivars ACCC50869 (AY463034) were downloaded from DDBJ/GenBank/EMBL for analysis. *Scale bar* indicates 0.01 substitutions/site

was the same as that of *P. eryngii* (AY368658), but differed from the labeled *P. nebrodensis* of China (Bai-Ling-Gu). The sequences in IGS1 and IGS2 were also different from those of the *P. nebrodensis* of China. ACCC 50656 was a real *P. eryngii* var. *ferula* in China (A-Wei-Mo). AFRL 6015, 6021, and 6022 were not related to *P. eryngii* var. *ferula* in China but were related to Bai-Ling-Gu from the results of the mon-mon mating (see Table 5), the di-mon mating (Table 6), and the sequences in ITS1 and IGS1 of rDNA.

The mon-mon mating ratio between AFRL 6016 (Bai-Ling-Gu) and AFRL 6021 (labeled A-Wei-Mo) was 100%, and the mated mycelia showed good growth (see Table 5). AFRL 6021 showed nearly the same results as those of AFRL 6016 in mon-mon mating and di-mon mating tests (data not shown). AFRL 6021 designated as A-Wei-Mo (*P. eryngii* var. *ferulae*) by the distributor, but it should be closely related to Bai-Ling-Gu. AFRL 6015, 6021, and 6022 fitted group 3 (*P. eryngii* var. *nebrodensis*) of the classification of Zhang et al. (2006) from some cultivation characteristics (data not shown).

The mon-mon mating ratio between AFRL 6016 (Bai-Ling-Gu) and CBS 100.82 (*P. eryngii* var. *eryngii*) was 65% (see Table 5). Bao et al. (2004a) and Zhang et al. (2006) did not succeed in the mating. We cultured for 7 weeks in the mating test, and we could not find clamp connections in the mycelia at the early stage of mating. Bao et al. (2004a) incubated for only 2 weeks. The period of culture may be the main reason for the difference of the results in the mating test.

The mated mycelia often grew slowly (see Table 5), but several grew well. Most of the latter ones (F_1) and the descendants (F_2) formed fruit bodies (Fig. 1O–R). The biological species of Bai-Ling-Gu should be *Pleurotus eryngii*, but Bai-Ling-Gu was different from *P. eryngii* var *eryngii* in several points, as follows. (1) The mon-mon mating ratio between Bai-Ling-Gu and *P. eryngii* var. *eryngii* was 65%. (2) Many of the mated mycelia grew slowly. (3) The di-mon mating ratio between Bai-Ling-Gu and *P. eryngii* var. *eryngii* was very low (see Table 6). (4) ITS1 and IGS1 sequences of rDNA were different (see Figs. 2, 3).

In the study of Hilber (1982), the ratios of mon-mon mating between *P. eryngii* var. *eryngii* and *P. eryngii* var. *nebrodensis*, *P. eryngii* var. *eryngii* and *P. eryngii* var. *ferulae*, and *P. eryngii* var. *ferulae* and *P. eryngii* var. *nebrodensis* were 18%–94%, 25%–53%, and 8%–45%, respectively. Zervakis and Balis (1996) divided the strains of *P. eryngii*

into three groups by their host plants (*Eryngium, Laserpitium*, and *Ferula*) and tested mon-mon and di-mon mating among the strains. The mating ratio of intragroup mon-mon mating was 94%–100%, that of intergroup mon-mon mating was 25%–88%, and that of di-mon mating was 95%–100%. The mating ratios of mon-mon mating described in Table 5 were in those ranges, but some ratios of di-mon mating shown in Table 6 were quite low. We have no clear explanation of the different mating ratio in di-mon matings, except the difference of the test strains.

The strains of *P. eryngii* var. *nebrodensis* of Italy were not uniform genetically. DS 391, 393, 504, and 506 were thought to be standard *P. eryngii* var. *nebrodensis*. They had lighter-colored caps, cream-colored lamellae, and large basidiospores, and the partial sequences of ITS1 and IGS1 were identical to those of the epitype. The stems of the fruit bodies were not excentric, but that might be caused by artificial cultivation. These strains originated from Sicily, Italy. DS 201 and DS 260 did not originate from Sicily, had pale apricot or pale brown lamellae, and smaller basidiospores, and the partial sequences of ITS1 and IGS1 were not identical to those of the epitype, but were nearly identical to the *P. eryngii* var. *eryngii-ferulae* complex. The standard strains of *P. eryngii* var. *nebrodensis* hardly mated with Bai-Ling-Gu and *P. eryngii* var. *ferulae*, but mated well with *P. eryngii* var. *eryngii* in the di-mon mating. Mating was frequent in mon-mon mating between *P. eryngii* var. *nebrodensis* and Bai-Ling-Gu (data not shown). Bai-Ling-Gu, *P. eryngii* var. *eryngii*, *P. eryngii* var. *ferulae*, and *P. eryngii* var. *nebrodensis* should be in the same species because propagation occurred among them. Their biological species should be *Pleurotus eryngii*, but there were distinct difference in mating behavior and the sequences of ITS1 and IGS1. The species could be divided into three groups in this study: Bai-Ling-Gu, *P. eryngii* var. *nebrodensis*, and *P. eryngii* var. *eryngii-ferulae* complex. The strains of *P. eryngii* var. *eryngii* and *P. eryngii* var. *ferulae* could be divided by di-mon mating, but there existed an intermediate strain (DS 201).

Therefore, Bai-Ling-Gu was different from Sicilian *P. nebrodensis*, including its type specimen, in this phylogenetic analysis. It evolved independently in China. Mao (2005) submitted that Bai-Ling-Gu was a cultured strain of *P. eryngii* var. *tuoliensis*. Jia and Qin (2006) summarized the history of cultivation of Bai-Ling-Gu and described that Bai-Ling-Gu was of the lineage of *P. eryngii* var. *tuoliensis*. However, the type specimen of *P. eryngii* var. *tuoliensis* could not be traced in the herbarium at Xinjiang Institute of Ecology and Geography, Chinese Academy of Science, where it was conserved. We suggest that *P. eryngii* var. *tuoliensis* can be a strong candidate for the scientific name for Bai-Ling-Gu.

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