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The biological species of oyster mushrooms (*Pleurotus* spp.) from Asia based on mating compatibility tests

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Abstract Mating compatibility of 25 *Pleurotus* species collected mainly from Asia was tested by either the Mon–Mon mating or the Di–Mon mating tests. The results showed 5 intersterility groups (*P. ostreatus*, *P. pulmonarius*, *P. cornucopiae*, *P. cystidiosus*, and *P. salmoneostramineus* complexes) and 7 independent interincompatible species (*P. calyptratus*, *P. corticatus*, *P. dryinus*, *P. eryngii*, *P. nebrodensis*, *P. smithii*, and *P. ulmarius*). The *P. ostreatus* complex includes *P. ostreatus*, *P. ostreatus* var. *columbinus*, *P. djamor*, and *P. flabellatus*. The *P. pulmonarius* complex has 7 taxa: *P. pulmonarius*, *P. eugrammus*, *P. eugrammus* var. *brevisporus*, *P. sajor-caju*, *P. sapidus*, *P. sp. florida*, and *P. opuntiae*. The *P. cornucopiae* complex includes its variant *P. cornucopiae* var. *citrinopileatus*. The *P. cystidiosus* complex includes *P. abalonus*. The *P. salmoneostramineus* complex includes 3 pink-colored mushrooms: *P. salmoneostramineus*, *P. ostreatoroseus*, and *P. rhodophyllus*. According to mating compatibility tests, 12 biological species were identified from among 25 *Pleurotus* species.

Key words Biological species · Intersterility group · Mating compatibility · *Pleurotus*

Introduction

Oyster mushrooms (*Pleurotus* spp.) comprise a major proportion of the edible mushrooms cultivated in the world. However, many questions about the taxonomy of *Pleurotus* spp. remain unresolved. In recent decades, several researchers have used biochemical and molecular techniques, as

well as mating compatibility tests, “Mon–Mon mating” or “Di–Mon mating”, to clarify the taxonomic and phylogenetic relationships among *Pleurotus* species.^{1–9}

The concept of the morphological species is dominant in the field of fungal taxonomy; most fungi are classified using morphological characteristics. However, morphological characteristics of higher fungi are inconsistent and unstable criteria because they are strongly influenced by the climate, cultivation substrate, and environmental conditions.¹⁰ As a result, different taxonomists have formed different conclusions regarding the taxonomic status of the same taxon based on morphological characters.

Another important taxonomic concept is that of the biological species. If two species are intercompatible, they are grouped as one biological species. Given the broad acceptance of this idea, mating compatibility tests have been used to evaluate the taxonomic identity of fungal species classified by morphological characters. In recent decades, several researchers have used mating tests for clarifying the biological species in basidiomycetous mushrooms.^{11–14} Vilgalys et al.⁵ identified 3 intersterility groups among North American *Pleurotus* collections. Petersen and Hughes⁴ reported 6 intersterility groups among 7 *Pleurotus* species. Recently, Zervakis and Balis⁹ studied the mating behaviors among 13 *Pleurotus* species originating from Europe, and divided them into 8 intersterility groups.

In this study, mating compatibility among 25 *Pleurotus* morphological species collected mainly from Asia was tested. The intersterility groups among these *Pleurotus* species are defined. With the support of the mating compatibility analysis, taxonomic relationships among these *Pleurotus* species are discussed.

Materials and methods

Organisms

Twenty-five *Pleurotus* dikaryotic strains representing different morphological species were used in this study (Table

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Table 1. Details of *Pleurotus* taxa tested in this study

Species	Strain no.	Geographic origin	Acquisition source
<i>Pleurotus abalonus</i> Han.	TD-200	Japan	MBTU
<i>Pleurotus calyptratus</i> (Lindbl.) Sacc.	IFO32795	Japan	IFO
<i>Pleurotus cornucopiae</i> (Paul. : Pers.) Roll.	MH00301	Japan	HOKUTO
<i>Pleurotus cornucopiae</i> var. <i>citrinopileatus</i> (Sing.) Ohira	0579	China	EFI
<i>Pleurotus corticatus</i> (Fr.) Quél.	580	China	EFI
<i>Pleurotus cystidiosus</i> Miller	4110	Japan	MBTU
<i>Pleurotus djamor</i> (Fr.) Boedijn	IFO32398	Japan	IFO
<i>Pleurotus dryinus</i> (Pers. : Fr.) Kumm.	ATCC 48595	Norway	ATCC
<i>Pleurotus eryngii</i> (DC. : Fr.) Quél	MH06062	China	HOKUTO
<i>Pleurotus eugrammus</i> (Mont.) Dennis	585	China	EFI
<i>Pleurotus eugrammus</i> var. <i>brevisporus</i> (Mont.) Dennis	574	China	EFI
<i>Pleurotus flabellatus</i> (Berk. et Br.) Sacc.	ATCC 62883	Unknown	ATCC
<i>Pleurotus nebrodensis</i> (Inz.) Sacc.	TD-021	China	MBTU
<i>Pleurotus ostreatoroseus</i> Sing.	96235	Brazil	ATCC
<i>Pleurotus ostreatus</i> (Jacq. : Fr.) Kumm.	TD-33	Japan	MBTU
<i>Pleurotus ostreatus</i> var. <i>columbinus</i> (Quél) Pilat	ATCC 36498	France	ATCC
<i>Pleurotus opuntiae</i> (Durieu : Leveille) Sacc.	ATCC 90202	India	ATCC
<i>Pleurotus pulmonarius</i> (Fr.) Quél	MH06043	Japan	HOKUTO
<i>Pleurotus rhodophyllus</i> Bres.	0595	China	EFI
<i>Pleurotus sajor-caju</i> (Fr.) Sing.	TD-991	Nepal	MBTU
<i>Pleurotus salmoneostramineus</i> Vass.	MH00504	Japan	HOKUTO
<i>Pleurotus sapidus</i> (Schulz.) Sacc.	0601	China	EFI
<i>Pleurotus smithii</i> Guzman	ATCC 46391	Mexico	ATCC
<i>Pleurotus</i> sp. <i>florida</i> (Fr.) Kumm.	TD-002	Thailand	MBTU
<i>Pleurotus ulmarius</i> (Bull. : Fr.) Quél	TD-003	Japan	MBTU

ATCC, American Type Culture Collection; EFI, Edible Fungi Institute, Shanghai Academy of Agricultural Science; HOKUTO, Hokuto Co. Ltd.; IFO, Institute of Fermentation, Osaka; MBTU, Laboratory of Microbial Biotechnology, Tottori University

1). Mycelia were cultured in plastic Petri dishes (Iwaki, 90 × 15mm) using 12ml potato dextrose agar (PDA) medium (Nissui Pharmaceutical). Plates were inoculated with a mycelial agar block (3 × 3 × 3mm) excised from stock culture, and incubated at 25°C in the dark.

Cultivation of fruit bodies

Fruit bodies of the test mushrooms were cultivated on sawdust substrate. The substrate was prepared by mixing beech (*Fagus crenata* Blume) sawdust and rice bran at a volumetric ratio of 3:1, and adjusting the moisture content with tap water to about 65% on wet basis. About 200g of the substrate was placed in a 300-ml polypropylene bottle. The bottle was plugged with a plastic cap and autoclaved at 120°C for 20min. After cooling the bottle to room temperature, the substrate was inoculated with the dikaryotic mycelia grown on PDA plates for 20–25 days at 25°C in the dark. After completing the spawn running, the surface of the mycelia in the culture bottle was scratched with a sterile spatula, and the old mycelia were removed. The culture bottle was then filled with sterile water, and the culture bottle was kept at 10°C for 24h. After decanting the water from the bottle, the culture was incubated at 20°C under continuous light (about 200lx) provided by fluorescent light tubes to initiate the development of fruit bodies. Fruit bodies were harvested after about 1 month of cultivation.

Preparation of monospore-derived isolates

The mature pilei of fruit bodies were cut off with a sterile knife, placed on sterile Petri dishes, and incubated at 10°C for 2–3 days to obtain spore print. The single basidiospore-derived monokaryotic isolates were prepared by adding 2ml sterilized water to the spore print in the Petri dish, shaking to prepare a basidiospore suspension, and transferring the suspension to test tubes at concentrations of 1×10^3 to 1×10^6 spores/ml. The spore concentrations were determined using a haemocytometer (Kayagaki Erika Kogyo) under magnification, and dilutions were made with water. The spore suspension (0.1ml) was mixed with 2ml of melted warm PDA agar at 50°C, and poured onto the surface of the PDA plate to prepare bilayer agar plates. The plates were incubated at 25°C, and the spore cells usually germinated after 3–4 days and formed monokaryotic mycelial colonies. When the colonies exceeded 2 mm in diameter, they were excised from the agar plates and planted on PDA slants to prepare spore isolate stocks. These slants were incubated for about 1 week at 25°C prior to use in the crossing experiments.

Mating type determination of monospore isolates

Test monokaryotic stocks were derived from each of the test dikaryotic stocks and the mating type of the test monokaryotic stocks was determined by crossing experiments.¹⁵ Twenty monospore isolates from each strain were crossed with each other at the center of the PDA plates. After incubating at 25°C for about 2 weeks, the clamp con-

nections of the mycelia growing in the contact zone between the two inocula were observed. By observing the mating behavior of the test crosses, each monokaryotic stock was designated as one of four types: *A1B1*, *A2B2*, *A1B2*, and *A2B1*. These stocks were used as the mating type tester monokaryons for each strain in mating compatibility tests.

Mating compatibility tests

Mating compatibility of the *Pleurotus* species except for *P. smithii* was tested in pairwise crosses of the monospore isolates of one species with one of the four tester stocks derived from another species (Mon–Mon mating test). The monokaryon of the tester stock and strain under consideration were inoculated 3–5 mm apart on a PDA plate, and incubated at 25°C for 7–10 days. Mycelium fragments taken from the contact zone between the paired mycelial colonies were examined under the light microscope and the pairing was scored as compatible if clamp connections were observed.

To identify mating compatibility characters of a dikaryotic strain of *P. smithii*, the dikaryon–monokaryon mating test (Di–Mon mating test) was performed between the test dikaryon and one of the four monokaryotic mating type tester stocks of another species in a procedure similar to the Mon–Mon mating tests. After the contact zone between the two inocula was formed, mycelium from the opposite margin of monokaryotic inoculum was excised and observed for the formation of clamp connections.

Results and discussion

Previous studies have used mating tests to identify intersterility groups among *Pleurotus* collections from North America and Europe. Three intersterility groups were identified among North American *Pleurotus* collections.⁵ Thirteen *Pleurotus* species in Europe were divided into eight intersterility groups.⁹ In another study, six intersterility groups containing seven *Pleurotus* species from different continents were identified.⁴ In the present study, mating compatibility tests revealed the existence of five intersterility groups (*P. ostreatus*, *P. pulmonarius*, *P. cornucopiae*, *P. cystidiosus*, and *P. salmoneostramineus*) and 7 independent interincompatible species (*P. calypttratus*, *P. corticatus*, *P. dryinus*, *P. eryngii*, *P. nebrodensis*, *P. smithii*, and *P. ulmarius*) among 25 *Pleurotus* species collected mainly from Asia (Fig. 1). The taxonomic status of five intersterility groups and a group of 7 interincompatible species is now discussed based on the biological species concept.

Pleurotus ostreatus complex

Pleurotus ostreatus is a typical oyster mushroom with a wide distribution in Asia, as well as in other continents. This mushroom is gray to brown in color, has a semicircular to mussel-shaped pileus, a lateral to eccentric stipe, and a white to gray spore print and monomitic hyphae system.⁹ *Pleurotus ostreatus* var. *columbinus* has a warm gray to steel

<i>P. ostreatus</i>																								
<i>P. ostreatus</i> var. <i>columbinus</i>	+	^D	<i>P. ostreatus</i> var. <i>columbinus</i>																					
<i>P. djamor</i>	+	+	<i>P. djamor</i>																					
<i>P. flabellatus</i>	+	+	<i>P. flabellatus</i>																					
<i>P. pulmonarius</i>	–	–	<i>P. pulmonarius</i>																					
<i>P. eugrammus</i>	–	–	<i>P. eugrammus</i>																					
<i>P. eugrammus</i> var. <i>brevisporus</i>	–	–	<i>P. eugrammus</i> var. <i>brevisporus</i>																					
<i>P. sajor-caju</i>	–	–	<i>P. sajor-caju</i>																					
<i>P. sapidus</i>	–	–	<i>P. sapidus</i>																					
<i>P. sp. florida</i>	–	–	<i>P. sp. florida</i>																					
<i>P. opuntiae</i>	–	–	<i>P. opuntiae</i>																					
<i>P. calypttratus</i>	–	–	<i>P. calypttratus</i>																					
<i>P. cornucopiae</i>	–	–	<i>P. cornucopiae</i>																					
<i>P. cornucopiae</i> var. <i>citrinopileatus</i>	–	–	<i>P. cornucopiae</i> var. <i>citrinopileatus</i>																					
<i>P. corticatus</i>	–	–	<i>P. corticatus</i>																					
<i>P. cystidiosus</i>	–	–	<i>P. cystidiosus</i>																					
<i>P. abalonus</i>	–	–	<i>P. abalonus</i>																					
<i>P. dryinus</i>	–	–	<i>P. dryinus</i>																					
<i>P. eryngii</i>	–	–	<i>P. eryngii</i>																					
<i>P. nebrodensis</i>	–	–	<i>P. nebrodensis</i>																					
<i>P. salmoneostramineus</i>	–	–	<i>P. salmoneostramineus</i>																					
<i>P. rhodophyllus</i>	–	–	<i>P. rhodophyllus</i>																					
<i>P. ostreatoroseus</i>	–	–	<i>P. ostreatoroseus</i>																					
<i>P. smithii</i>	– ^D	– ^D	<i>P. smithii</i>																					
<i>P. ulmarius</i>	–	–	<i>P. ulmarius</i>																					

Fig. 1. The results of Mon–Mon matings and Di–Mon matings among *Pleurotus* taxa. Plus signs, compatible Mon–Mon matings; minus signs, incompatible Mon–Mon matings; superscript *D*, Incompatible Di–Mon matings

brown pileus and only grows in central and western Europe.⁹ Despite their geographic isolation, Zervakis and Balis⁹ reported in 1996 that these two species showed intercompatibility. We confirmed this result in the present study.

Pleurotus djamor is characterized by its white color, spathulate to flabelliform pileus, a lateral and short stipe, pale cream spore print, and a dimitic hyphae system.¹⁶ In 1988, Neda et al.¹⁷ reported that an isolate of *P. djamor* from Okinawa, Japan was compatible with *P. salmoneostramineus*. On the other hand, Petersen and Hughes⁴ reported in 1993 that a Mexican strain of *P. djamor* is incompatible with *P. salmoneostramineus* and *P. ostreatoroseus* while the latter two taxa are intercompatible. However, the same authors have reported in a more recent paper that *P. djamor* shows mating compatibility with *P. salmoneostramineus*, *P. ostreatoroseus*, and *P. opuntiae*.¹² The results of mating tests in the present study confirm the former report by Petersen and Hughes.⁴ In addition, a Mexican isolate of *P. djamor* was presented as a member of the *P. ostreatus* complex based on mating test results by Peterson.¹⁸ Combining this finding with our results (Table 2), we suggest that some *Pleurotus* isolates under the name of “*djamor*” are closely related to *P. ostreatus*, but we do not exclude the possibility of prior misidentification of this taxon.

There are also problems with the taxonomy of *P. flabellatus*, which is described as a synonym of *P. djamor*.¹⁶ According to Zervakis and Balis,⁹ *P. flabellatus* is an independent intersterility species and is incompatible with *P. ostreatus*, *P. opuntiae* (a variant of *P. djamor* by Petersen¹²), and other taxa belonging to the *P. pulmonarius* complex. In this study, ATCC 62883 of *P. flabellatus* was compatible with *P. ostreatus* var. *columbinus* and *P. djamor*, and partially compatible with *P. ostreatus*. These results suggest that this strain of *P. flabellatus* might be a biological species in the *P. ostreatus* complex. However, the taxonomic status of *P. flabellatus* is still unclear because it might contain misidentified *P. flabellatus* strains isolated by different researchers.

Pleurotus pulmonarius complex

Seven species originating from Japan, China, Thailand, and India including *P. pulmonarius*, *P. eugrammus*, *P. eugrammus* var. *brevisporus*, *P. sajor-caju*, *P. sapidus*, *P. sp. florida*, and *P. opuntiae* were found to be intercompatible in our mating compatibility tests.

Pleurotus pulmonarius is a common *Pleurotus* taxon. It occurs in Europe, North America, and Asia and is generally characterized by a spathulate-shaped, brown pileus, a lateral to subcentral stipe, cream to buff spore print, and a monomitic hyphae system.⁹ Various morphological characters distinguish taxa in this group. *Pleurotus eugrammus* has white to grayish color and flabelliform-shaped pileus, and a white spore print.¹⁶ It can be distinguished from var. *brevisporus* by a larger spore size.¹⁶ *Pleurotus sapidus* is described by a white to yellow pileus, lilac spore print, and a monomitic hyphae system.¹⁹ *Pleurotus opuntiae* has a

cream spathulate-shaped pileus, lateral to subcentral stipe, a white spore print, and a dimitic hyphae system.⁹ *Pleurotus sajor-caju* and *P. sp. florida* are the most common commercial taxa and both have very similar morphological characters with *P. pulmonarius* when grown in our laboratory.

Several taxa in this group are always confused with the typical oyster mushroom *P. ostreatus*. The notable example is the difficulty in distinguishing *P. pulmonarius* from *P. ostreatus* based on morphological characters.²⁰ Although Zervakis and Balis⁹ point out that *P. pulmonarius* has a lighter pileus color, slightly larger basidiospores, a thinner pileus, and faster growth rates than that of *P. ostreatus*, it is difficult to separate the two species based on these differences. In this study, we suggest that *P. ostreatus* and *P. pulmonarius* are two different species based on their interincompatibility. Previous studies^{4,9,21} also support this conclusion.

Another example is *P. sapidus*. Previous studies^{22–24} classify *P. sapidus* as a conspecies with *P. ostreatus*. However, Zervakis and Balis⁹ demonstrated that strain ATCC 30986 of *P. sapidus* is compatible with *P. pulmonarius*, but incompatible with *P. ostreatus*. Additional studies using starch-gel electrophoresis⁸ and restriction fragment length polymorphism (RFLP) analysis³, as well as the mating test results from this study also support this result.

The classifications of strains of *P. sajor-caju* and *P. sp. florida* are very questionable. The type *P. sajor-caju* wild strain is described as having a ring-like veil, an amphitritic hyphae system, and nearly no subhymenium.²⁵ However, the commercial strain of *P. sajor-caju* cultivated under laboratory conditions in this study shows very similar morphology with *P. pulmonarius*. Zervakis and Balis⁹ have also reported this result, and the authors agree with their observation. *Pleurotus sajor-caju* represents a group of strains of *P. pulmonarius* that are widely cultivated in southern and eastern Asia.

The application of the name “*florida*” seems to cause the most confusion in the taxonomy of *Pleurotus*.^{5,26} Bresinsky et al.²⁶ and Hilber²¹ conclude that strains under the name of *florida* originate from two species: *P. ostreatus* and *P. pulmonarius*. For this reason, *Pleurotus* strains under the name of *florida* are reported to be compatible with either *P. ostreatus*²² or *P. pulmonarius*.¹⁰ Buchanan²⁰ suggested that *Pleurotus* taxa should not be designated as *florida* in the scientific literature, and mating tests would be an effective way to clarify the identity of *Pleurotus* strains named *florida*.

On the other hand, *P. opuntiae* has been reported to be a morphological variant within the *P. djamor* intersterility group including *P. salmoneostramineus* based on examination of American and New Zealand strains.¹² However, Zervakis and Balis⁹ showed that a Greek isolate of *P. opuntiae* is an independent intersterility species (incompatible with *P. flabellatus*, *P. pulmonarius*, *P. sajor-caju*, and *P. sapidus*). In the present study, an Indian isolate (ATCC 90202) of *P. opuntiae* was compatible with all members of the *P. pulmonarius* intersterility group. The taxonomic identity of *P. opuntiae* needs to be studied further, after collecting more isolates.

The above-mentioned taxonomic discrepancies within the *Pleurotus* taxa are caused not only by misidentification and misapplication, but also by the lack of effective identification criteria and limited collections of examined isolates. These problems also exist within the taxa described below. The authors consider that the resolution of these problems is dependent on the comprehensive application of mating tests and molecular techniques to analyze extensive collections from various climates and areas worldwide.

Pleurotus cornucopiae complex

A Japanese strain of *P. cornucopiae* is compatible with a Chinese strain of *P. cornucopiae* var. *citrinopileatus*. The former has a distribution in Europe and Asia, is characterized by a yellow pileus, a branched subcentral stipe, a dimitic hyphae system, and a distinctive purple spore print.^{9,27} The latter mainly occurs in the eastern Asia, and has similar morphological characters with the former. However, it can be differentiated from *P. cornucopiae* based on the following morphological traits: the brighter yellow color of the pileus, smaller basidiospores, lighter spore print color, an abundance of cheilocystidia, and monomitic trama.²² According to comparisons made by Zervakis and Balis⁹ of artificial cultivation characters of these two taxa, the morphological differences are very small, and the hyphae system of *P. cornucopiae* var. *citrinopileatus* was reported to also be dimitic. Although Singer²⁸ described *P. citrinopileatus* as a separate species, Ohira²⁷ reduced *P. citrinopileatus* to varietal status under *P. cornucopiae* and named it *P. cornucopiae* var. *citrinopileatus* according to the morphological similarities and intercompatibility. The mating tests in this study also support the designation of these two taxa as the same species.

Pleurotus cystidiosus complex

The Japanese strain of *P. cystidiosus* and *P. abalonus* were intercompatible. *Pleurotus cystidiosus* is the type species in the *Coremiopleurotus* section of genus *Pleurotus*⁹ and is primarily characterized by formation of asexual coremia, long basidiospores, and very low growth rates of mycelia.²⁸

The related taxa of *P. cystidiosus* are *P. abalonus* and *P. smithii*. The former was first reported to grow on *Acer* sp. in forests of Taiwan and was treated as a new distinct species.²⁹ It is distinguished from *P. cystidiosus* by the small basidiospores and cheilocystidia.³⁰ However, recent studies tend to consider *P. abalonus* to merely be a geographical variant.²⁸ The authors support assorting *P. abalonus* and *P. cystidiosus* into a common biological species based on intercompatibility found in previous crossing tests^{21,26,31} and in this study. The latter species was first found in Mexico³⁰ and can be differentiated from *P. cystidiosus* by the rare pleurocystidia, the long and subcylindric cheilocystidia, short conidiophores, and the presence of chlamydospore.³⁰ Although *P. smithii* has morphological similarity with *P. cystidiosus*,³⁰ it has so far been reported to be incompatible with *P. cystidiosus*.⁹ In this study, the Di-Mon mating tests

between a dikaryon strain (ATCC 46391) of *P. smithii* and the monokaryotic isolates from *P. abalonus* and *P. cystidiosus* showed negative results (Fig. 1).

Pleurotus salmoneostramineus complex

This complex includes three pink mushrooms: *P. salmoneostramineus* (from Japan), *P. rhodophyllus* (from China), and *P. ostreatoroseus* (from Brazil). *Pleurotus rhodophyllus* is characterized by salmon pink coloration and spatula-shaped pileus, lateral to eccentric stipe, and pink spore print.³² *Pleurotus rhodophyllus* is reportedly found only in Yunnan Province of China.³² *Pleurotus salmoneostramineus* can be distinguished from *P. rhodophyllus* by lighter pink color and the absence of pleurocystidia.¹⁸ The morphology of the cultivated fruit body of *P. salmoneostramineus* has a spatula-shaped pileus and a lateral stipe, while *P. ostreatoroseus* has flabellate- to shell-shaped pileus and a nearly centric stipe. Petersen¹² considers these two taxa to be congruent and belonging to the *P. djamor* intersterility group.

Interincompatible species

The remaining seven strains shown in Fig. 1 were incompatible with each other: *P. calypttratus* (from Japan), *P. corticatus* (from China), *P. dryinus* (from Norway), *P. eryngii* (from China), *P. nebrodensis* (from China), *P. smithii* (from Mexico), and *P. ulmarius* (from Japan). Therefore, all of these species are treated as one independent intersterility group.

Among these taxa, *P. calypttratus* and *P. dryinus* have been shown to be a single intersterility species by Zervakis and Balis.⁹ Another two taxa, *P. corticatus* and *P. ulmarius*, have not been subjected to mating tests.

The taxonomic relationships of *P. eryngii* and *P. nebrodensis* were examined. The former is a popular cultivated mushroom in Japan. The latter has been rapidly developed for cultivation in China, and is sometimes treated as a variant of *P. eryngii* and is sometimes confused with another widely cultivated mushroom *Pleurotus ferulae*. Zervakis and Balis⁹ concluded that these three species have partial compatibility with one another. However, we have shown that *P. eryngii* and *P. nebrodensis* are incompatible in our mating tests. The relationships among these three taxa need further study.

The establishment of the intersterility group provided useful information for the analysis of taxonomic relationships among *Pleurotus* species. All intercompatible species are considered to be one intersterility group. The name of the type species in the intersterility group is designated using the name of the intersterility group, and the other species are treated as subspecies. When one species is demonstrated to be interincompatible with all other species, it is designated as an independent intersterility group (biological species).

In conclusion, 12 intersterility groups (biological species) have been established among 25 *Pleurotus* morphological

Table 2. The intersterility groups in the *Pleurotus* taxa based on mating compatibility tests

Species	Biological subspecies	Intersterility groups
<i>P. ostreatus</i>	<i>P. ostreatus</i> var. <i>columbinus</i> <i>P. djamor</i> <i>P. flabellatus</i>	I
<i>P. pulmonarius</i>	<i>P. eugrammus</i> <i>P. eugrammus</i> var. <i>brevisporus</i> <i>P. sajor-caju</i> <i>P. sapidus</i> <i>P. sp. florida</i> <i>P. opuntiae</i>	II
<i>P. calyptratus</i>		III
<i>P. cornucopiae</i>	<i>P. cornucopiae</i> var. <i>citrinopileatus</i>	IV
<i>P. corticatus</i>		V
<i>P. cystidiosus</i>	<i>P. abalonus</i>	VI
<i>P. dryinus</i>		VII
<i>P. eryngii</i>		VIII
<i>P. nebrodensis</i>		IX
<i>P. salmoneostramineus</i>	<i>P. rhodophyllus</i> <i>P. ostreatoroseus</i>	X
<i>P. smithii</i>		XI
<i>P. ulmarius</i>		XII

species mainly originating from China and Japan (Table 2). In previous studies of *Pleurotus* taxonomy, samples had been mainly collected from North America and Europe, but few from Asia. However, Asia is also a main genetic resource area of edible mushrooms, and many *Pleurotus* species are widely distributed throughout the Asian continent. The results in our study provide taxonomic data on *Pleurotus* species in Asia.

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