Exploitation of Wild Pleurotus Germplasm: Domestication, Molecular Characterization, and Interspecific Hybrid Breeding

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Abstract

Pleurotus species harbor rich wild germplasm, but commercial cultivation relies on few strains, narrowing genetic diversity. This study domesticated wild Pleurotus djamor (HP), P. pulmonarius (FX), P. ostreatus (CP, CPH) and a commercial P. eryngii (XB), evaluated growth and substrate utilization, performed monokaryotic crossbreeding, and analyzed hybrid transcriptomes. FX showed the fastest mycelial growth and shortest substrate colonization time, while XB was slowest; carbon and nitrogen source preferences were strain-specific, with organic nitrogen generally superior. FX, HP, and XB were selected as parents. From 83 monokaryons and 372 crosses, HP×FX yielded the highest hybridization success; selected hybrids were confirmed by molecular methods. HP×XB hybrids showed severe fruiting abnormalities, whereas HP×FX hybrids produced normal, morphologically distinct fruiting bodies. Transcriptome analysis placed hybrid expression between parents but biased toward FX; enriched functions in hybrids included oxidoreductase and secondary metabolism, while pathway enrichment (endocytosis, cell cycle, proteasome, nucleocytoplasmic transport) mirrored parental differences. These results demonstrate the value of wild Pleurotus germplasm and targeted crossbreeding for creating genetically and phenotypically novel cultivars.

Keywords: Pleurotus; wild germplasm; domestication; monokaryon crossbreeding; hybridization; mycelial growth; substrate utilization; transcriptome analysis; gene expression bias; oxidoreductase; secondary metabolism; cultivar improvement.

1. Introduction

The genus *Pleurotus* (oyster mushrooms) represents one of the most economically important groups of edible fungi worldwide. Due to their high nutritional value, diverse bioactive compounds, rapid growth rate, and ability to utilize a wide range of lignocellulosic substrates, *Pleurotus* species have become the second most widely cultivated mushrooms globally, following *Agaricus bisporus* (Royse et al., 2017; Sánchez, 2009). In recent years, increasing global demand for functional foods and sustainable agricultural practices has further accelerated the expansion of *Pleurotus* cultivation and commercialization (Wasser, 2010).Beyond their economic importance, *Pleurotus* species play a critical ecological role as white-rot fungi, capable of efficiently degrading lignin, cellulose, and hemicellulose through extracellular enzymatic systems, including laccase and peroxidases (Kirk & Cullen, 1998). This metabolic versatility enables their cultivation on a wide range of agricultural and forestry residues, such as straw, sawdust, and cottonseed hulls, contributing to waste valorization and environmental sustainability (Pandey et al., 2018). In addition, *Pleurotus* mushrooms are widely recognized as nutrient-dense functional foods, containing high-quality proteins, essential amino acids, vitamins, and minerals, along with bioactive compounds such as β-glucans and lovastatin, which exhibit immunomodulatory, antioxidant, and hypolipidemic properties (Mattila et al., 2001; Alam et al., 2009).Despite these advantages, the sustainable development of the *Pleurotus* industry is increasingly constrained by the limited genetic diversity of commercially cultivated strains. Most production systems rely on a narrow pool of elite cultivars, resulting in reduced adaptability to environmental stresses, vulnerability to diseases, and limited potential for yield and quality improvement (Zhang et al., 2019). Therefore, expanding the genetic base of breeding materials has become a critical priority.

Wild germplasm resources of *Pleurotus* represent a valuable reservoir of genetic diversity, harboring traits such as stress tolerance, substrate adaptability, and unique morphological and biochemical characteristics. The systematic collection, domestication, and evaluation of wild strains provide essential raw materials for breeding programs (Chang & Miles, 2004). Through domestication, wild strains can be adapted to artificial cultivation conditions, while retaining their advantageous genetic traits. Sexual reproduction in *Pleurotus*, governed by a tetrapolar mating system controlled by two independent mating-type loci (A and B), plays a fundamental role in generating genetic diversity (Casselton & Kües, 2007). Only monokaryotic strains with different alleles at both loci are compatible, leading to the formation of dikaryotic mycelia capable of producing fruiting bodies. This complex mating system, characterized by high allelic diversity, enhances recombination potential and provides a strong genetic basis for breeding (Kües, 2015).

Among the available breeding strategies, monospore hybridization has proven to be one of the most effective approaches for developing improved *Pleurotus* strains. This method involves the isolation of single basidiospores to obtain genetically distinct monokaryons, followed by controlled mating between compatible strains to generate dikaryotic hybrids with desirable agronomic traits (Royse, 2010). Compared with traditional selection methods, monospore hybridization offers greater precision and efficiency in combining favorable genetic characteristics. In recent years, advances in molecular biology and genomics have further enhanced breeding strategies in *Pleurotus*. Molecular markers, mating-type gene identification, and genome-based analyses have improved the efficiency of parental selection and hybrid evaluation. However, despite these technological advances, the integration of wild germplasm domestication with systematic hybrid breeding remains insufficiently explored.

Therefore, the present study aims to integrate wild germplasm domestication with monospore hybridization to broaden the genetic base of *Pleurotus* breeding. Specifically, this study focuses on (i) the domestication and evaluation of wild

Pleurotus strains, (ii) the characterization of mating-type diversity among parental strains, and (iii) the development of hybrid strains with improved agronomic performance. The outcomes of this research are expected to provide both theoretical insights and practical strategies for the sustainable development of the *Pleurotus* industry.

2. Materials and Methods

2.1 Materials

2.1.1 Fungal strains

Five representative *Pleurotus* strains (*Pleurotus djamor*, *Pleurotus pulmonarius*, *Pleurotus ostreatus*, and *Pleurotus eryngii*) were used in this study. Wild isolates were collected from the forest area of Jilin Agricultural University, while additional strains were obtained from culture collections and commercial sources.

Species identification and strain handling followed standard taxonomic procedures for basidiomycetes (Hibbett et al., 2014; Vilgalys & Sun, 1994).

2.1.2 Culture media and chemical formulations

PDA medium consisted of potato (200 g L^{-1}), glucose (20 g L^{-1}), agar (20 g L^{-1}), and distilled water, adjusted to pH 6.0–6.5 (Hawksworth et al., 2008).
PDB medium contained potato (200 g L^{-1}) and glucose (20 g L^{-1}).

Basal medium included KH_2PO_4 (3 g L^{-1}), $MgSO_4$ (1.5 g L^{-1}), and agar (18 g L^{-1}).

Carbon and nitrogen utilization media were prepared according to standard fungal physiological methods, where single carbon or nitrogen sources were substituted while maintaining basal nutrient composition (Chang & Miles, 2004). The cultivation substrate formulation followed established oyster mushroom cultivation practices using lignocellulosic agricultural residues (Royse et al., 2017).

2.1.3 Reagents

All reagents were analytical grade. DNA extraction, PCR reagents, and electrophoresis chemicals were used according to manufacturer instructions. Congo red and KOH solutions were prepared following standard fungal microscopy protocols (Ainsworth, 1976).

2.1.4 Instruments and equipment

Microscopic observations were conducted using ZEISS compound microscopes and Hitachi scanning electron microscopy systems. Molecular analyses used standard PCR thermocyclers, electrophoresis systems, and NanoDrop spectrophotometers. All equipment followed standard mycological laboratory protocols (Sambrook & Russell, 2001).

2.2 Methods

2.2.1 Sample collection, isolation, and morphological observation

2.2.1.1 Field collection and documentation

Fruiting bodies of Pleurotus spp. were collected from natural environments. Ecological parameters, host substrate, and habitat conditions were recorded following standard fungal biodiversity survey methods (Mueller et al., 2004). Macroscopic traits were documented using high-resolution photography.

2.2.1.2 Isolation and purification

Tissue isolation was performed under sterile conditions following standard fungal culture techniques (Stamets, 2000). Internal tissues (3–5 mm) were excised and inoculated onto PDA medium at 25 °C. Pure cultures were obtained through repeated subculturing (2–3 transfers).

2.2.1.3 Specimen preservation

Specimens were dried at 50 °C or preserved using silica gel for molecular analysis following herbarium preservation protocols for macrofungi (Ainsworth, 1976).

2.2.1.4 Microscopic observation

Microscopic structures were examined using 5% KOH mounts and Congo red staining. Basidiospore measurements followed standard taxonomic methods (Singer, 1986). Twenty to thirty spores per specimen were measured for statistical accuracy.

2.2.2 Molecular identification (ITS sequencing)

Genomic DNA was extracted using a commercial fungal DNA extraction kit following manufacturer protocols (Sambrook & Russell, 2001). DNA quality was evaluated using agarose gel electrophoresis and spectrophotometry.

The ITS region was amplified using primers ITS1 and ITS4 (White et al., 1990), widely accepted as universal fungal barcoding markers (Schoch et al., 2012).

PCR amplification followed standard thermocycling conditions:
94 °C for 5 min; 35 cycles of 94 °C for 30 s, 54 °C for 30 s, 72 °C for 1 min; final

extension at 72 °C for 10 min. PCR products were analyzed by agarose gel electrophoresis and sequenced commercially.

2.2.3 Mycelial growth rate determination

Mycelial growth was measured using the cross-intersection method on PDA plates incubated at 25 °C. Growth rate was calculated as colony diameter increase per unit time, following fungal physiology standards (Chang & Miles, 2004).

2.2.4 Single-factor experiments

Carbon and nitrogen utilization tests were conducted using a completely randomized design. Each treatment included three replicates. Data were analyzed using one-way ANOVA, and significance was determined at $p < 0.05$ (Gomez & Gomez, 1984).

2.2.5 Cultivation (spawn run) experiment

Substrate preparation and bag cultivation followed standard oyster mushroom production methods (Stamets, 2000; Royse et al., 2017). Sterilized substrate bags (650 g) were inoculated with solid or liquid spawn and incubated at 25 ± 1 °C. Full colonization time was recorded when substrate was fully colonized.

2.2.6 Fruiting induction

Fruiting conditions were established by transferring colonized bags into controlled environments with ~90% relative humidity. Bag openings were adjusted to regulate CO_2 concentration and promote basidiocarp development (Chang & Miles, 2004).

3. Results and Analysis

3.1 Morphological characteristics of wild *Pleurotus* strains

3.1.1 *Pleurotus djamor* (Rumph. ex Fr.) Boedijn

Fruiting bodies of *Pleurotus djamor* were typically clustered or fasciculate, with thin, soft, and elastic texture. Fresh basidiocarps exhibited pink to pale red coloration, gradually fading to light pink or pale orange during maturation. The pileus diameter ranged from 3–10 cm, with a thickness of 1–3 mm. Upon drying, the coloration changed to light brown. The hymenial surface was smooth and pinkish to light orange.

The pileus was fan-shaped, semicircular, or shell-shaped, with a smooth surface and thin, often undulate margins. The stipe was lateral or nearly absent, short, and white to pale pink in color. Basidiospores were hyaline, ellipsoid to subglobose, thin-walled, and smooth. Ecologically, this species is a typical wood-decaying fungus, mainly occurring on decayed hardwood logs in warm and humid environments. It is widely distributed in southern and southwestern China, including Yunnan, Guangxi,

Guangdong, Fujian, and Hainan, and is also reported from tropical and subtropical regions worldwide.

3.1.2 *Pleurotus pulmonarius*

Basidiocarps of *P. pulmonarius* were clustered, with medium-thin, flexible texture. The pileus was initially white to milky white, gradually turning light gray or pale brown with maturation, and becoming slightly darker in older specimens. The pileus diameter ranged from 4–12 cm, with a thickness of 2–5 mm. The hymenium was smooth and white to cream-colored.

The pileus was fan-shaped, semicircular, or reniform, with a smooth to slightly velvety surface and thin, wavy margins. The stipe was lateral or eccentric, short, and white to pale cream. Basidiospores were hyaline, ellipsoid to broadly ellipsoid, measuring approximately 7–10 × 3–4 μm.

This species is a saprotrophic wood-decaying fungus widely distributed in temperate and subtropical regions and shows strong ecological adaptability and heat tolerance.

3.1.3 *Pleurotus ostreatus* (Jacq.) P. Kumm.

Basidiocarps of *P. ostreatus* were typically clustered, with relatively thick and soft texture. Pileus coloration varied from gray-white to dark gray or gray-brown depending on maturity. The pileus diameter ranged from 5–15 cm, with a thickness of 3–8 mm. The hymenial surface was smooth and white to pale gray.

The pileus was shell-shaped or fan-shaped with thin margins. The stipe was lateral or eccentric and short, sometimes absent. Basidiospores were hyaline, ellipsoid to subglobose, smooth-walled.

This species is a typical lignicolous saprotroph widely distributed across China and globally, and represents one of the most important cultivated edible mushrooms worldwide.

3.2 Phylogenetic analysis of strains

ITS-PCR amplification of genomic DNA from four parental strains produced clear single bands with no nonspecific amplification (Fig. 2.5). The amplified ITS fragments were approximately 600–700 bp in length, consistent with expected fungal ITS regions, indicating reliable amplification quality suitable for sequencing.

Phylogenetic analysis based on ITS sequences and BLAST alignment revealed that all strains were clearly separated into two major clades. *Pleurotus eryngii* formed an independent lineage with high bootstrap support (99%), clustering closely with reference sequences from GenBank. In contrast, *P. djamor*, *P. pulmonarius*, and *P.*

ostreatus grouped into a second clade with relatively close genetic relationships but distinct species-level divergence.

These results confirm clear genetic differentiation among the tested strains and provide a solid molecular basis for subsequent interspecific breeding and germplasm utilization.

3.3 Mycelial growth rate of different strains

Significant differences in colony growth rates were observed among strains under identical culture conditions (Table 2-2). *P. pulmonarius* (FX) exhibited the highest growth rate (17.19 ± 0.19 mm d^{-1}), significantly higher than other strains ($P < 0.05$). *P. djamor* (HP) also showed strong growth (16.03 ± 0.21 mm d^{-1}).

P. ostreatus strains CP and CPH exhibited moderate growth rates (14.24 ± 0.19 and 13.24 ± 0.17 mm d^{-1}, respectively), with no significant difference between them. The lowest growth rate was observed in *P. eryngii* (XB), at 10.69 ± 0.19 mm d^{-1}.

These results indicate clear interspecific variation in vegetative growth ability, reflecting physiological and genetic differences among strains.

3.4 Effects of carbon and nitrogen sources on mycelial growth

3.4.1 Carbon source utilization

Carbon sources significantly influenced mycelial growth, and responses varied among strains (Table 2-3). HP showed the highest growth rate on dextrin (25.81 ± 2.16 mm d^{-1}), while FX performed best on sucrose (25.10 ± 1.71 mm d^{-1}). CP and CPH exhibited maximum growth on soluble starch (24.64 ± 0.55 and 23.15 ± 0.58 mm d^{-1}, respectively).

In contrast, the control treatment (CK) showed relatively lower growth, indicating that exogenous carbon supplementation significantly enhanced mycelial development. Notably, glucose did not promote growth in most strains and even showed inhibitory effects in some cases, suggesting strain-specific carbon metabolism preferences.

3.4.2 Nitrogen source utilization

Nitrogen sources also significantly affected mycelial growth (Table 2-4). Organic nitrogen sources, particularly soybean peptone and yeast extract, generally promoted higher growth rates compared with inorganic ammonium sulfate.

HP showed the highest growth on yeast extract (19.89 ± 4.30 mm d^{-1}), while FX, CP, and CPH showed optimal growth on soybean peptone. In contrast, ammonium sulfate consistently inhibited mycelial growth across all strains, indicating poor utilization of inorganic nitrogen forms.

These results suggest that Pleurotus strains preferentially utilize complex organic nitrogen sources for vegetative growth.

3.5 Spawn run time (bag colonization period)

Significant differences in full colonization time were observed among strains (Table 2-5). FX showed the shortest spawn run time (20.9 ± 1.79 d), followed by CPH (22.7 ± 1.16 d) and CP (24.2 ± 1.14 d). HP required 25.7 ± 2.11 d, while XB exhibited the longest colonization period (29.4 ± 2.22 d).

Overall, spawn run duration was consistent with mycelial growth rate, confirming that vegetative growth ability strongly influences substrate colonization efficiency.

3.6 Fruiting performance and biological efficiency

3.6.1 First flush yield and biological efficiency

Significant differences in yield performance were observed among strains (Table 2-6). XB produced the highest first flush fresh weight (129.97 ± 4.34 g), significantly higher than all other strains, with a biological efficiency of 57%.

The remaining strains (HP, FX, CP, and CPH) showed no significant differences in yield, ranging from 87.02 to 91.65 g, with biological efficiencies between 38% and 40%.

These results indicate that although some strains showed strong vegetative growth, this did not necessarily translate into higher yield performance.

3.6.2 Fruiting body development

All strains successfully formed fruiting bodies under controlled environmental conditions (Fig. 2.6–2.9). Morphological characteristics of cultivated basidiocarps were consistent with wild-type descriptions, confirming successful domestication and cultivation stability

2.3.1 Morphological characteristics of wild fungal strains

Pleurotus djamor (Rumph. ex Fr.) Boedijn

Basidiocarps are clustered or fasciculate, thin, soft, and elastic when fresh. The pileus is pink to light red in early stages, becoming pale pink to light orange at maturity. The fruiting bodies measure 3–10 cm in diameter and 1–3 mm in thickness, turning light brown when dried. The hymenial surface is smooth and pink to pale orange. The pileus is fan-shaped, semicircular, or shell-shaped with a smooth surface and thin, often wavy margins. The stipe is lateral or nearly absent, short, and white to pale pink. Basidiospores are hyaline, ellipsoid to subglobose, thin-walled, and smooth.

Ecologically, it is a wood-decaying fungus found on decayed hardwood in warm and humid environments. It is widely distributed in southern and southwestern China (Yunnan, Guangxi, Guangdong, Fujian, Hainan) and in tropical and subtropical regions worldwide.

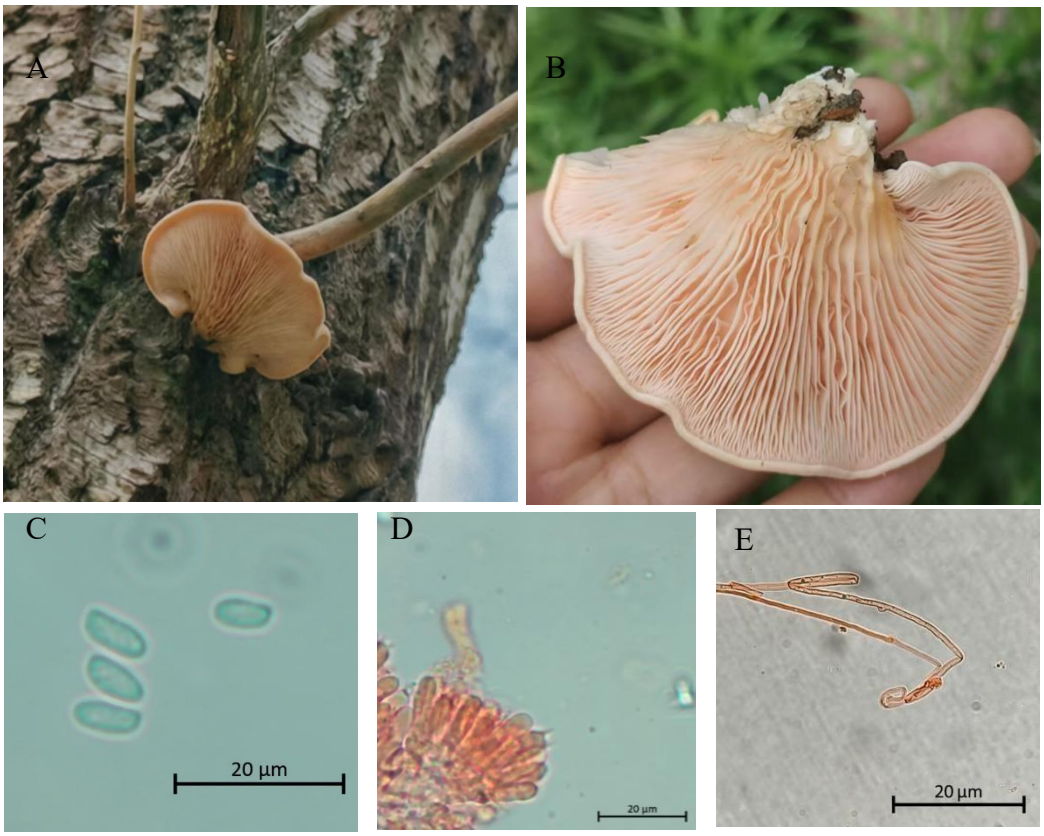

Figure 2.1 *Pleurotus djamor* (Rumph. ex Fr.) Boedijn
A: Habitat photograph; B: Basidiocarp; C: Basidiospores; D: Basidia; E: Hyphae with clamp connections.

Basidiocarps: Typically clustered or fasciculate, with medium flesh that is thin and flexible. The pileus is white to milky white at the early stage, gradually turning light gray or pale brown with growth, and becoming slightly darker at maturity. The basidiocarp diameter is usually 4–12 cm, with a thickness of about 2–5 mm. When dried, it becomes light yellowish-brown. The hymenial surface is smooth, white to pale cream in color.

Pileus: Fan-shaped, semicircular, or reniform. The surface is smooth or slightly velvety, with thin margins that are inrolled when young and become expanded and wavy at maturity.

Stipe: Lateral or eccentric, short, and sometimes indistinct; white to pale milky white in color with a firm texture.

Basidiospores: Hyaline, ellipsoid to broadly ellipsoid, thin-walled, smooth, measuring approximately 7–10 μm × 3–4 μm.

Ecology: A wood-decaying fungus mainly found on decayed hardwood logs, fallen wood, or rotten wood. It can also occur on cultivated substrates. It prefers warm environments and shows relatively high heat tolerance.

Distribution in China: Widely distributed in southern and central China, including Yunnan, Sichuan, Guangxi, Guangdong, Fujian, and Hunan.

Global distribution: Occurs in temperate and subtropical regions worldwide, including Asia, Europe, Africa, and the Americas, showing strong ecological adaptability.

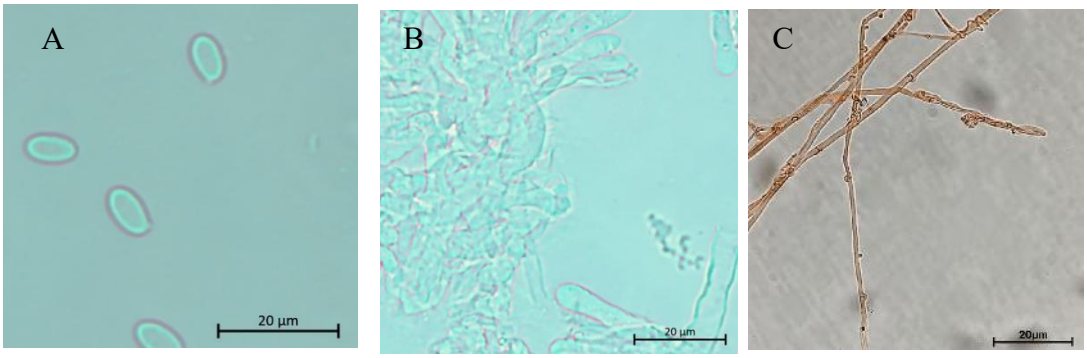

Figure 2.2 *Pleurotus pulmonarius* (Fr.) Quél.

A: Basidiospores; B: Basidia; C: Mycelium with clamp connections.

Pleurotus ostreatus (Jacq.) P. Kumm.

Basidiocarps: Typically clustered or fasciculate, with thick and soft flesh. The pileus shows considerable color variation, commonly gray-white, gray-brown, or dark gray. The pileus diameter is generally 5–15 cm, with a thickness of about 3–8 mm. The hymenial surface is smooth, white to pale gray.

Figure 2.3 Field collection photograph of *Pleurotus ostreatus*
Fig.2.3 *Pleurotus ostreatus* wild collection photo

Pileus: Shell-shaped or fan-shaped, with a smooth or slightly velvety surface and thin margins.

Stipe: Lateral or eccentric, short, sometimes indistinct, white in color.

Basidiospores: Hyaline, ellipsoid to subglobose, thin-walled, and smooth-surfaced.

Ecology: A wood-decaying fungus commonly found on decayed hardwood logs, fallen wood, and rotting timber. It is a typical lignicolous saprotroph (Kirk et al., 2008; Stajich et al., 2009)..

Distribution in China: Widely distributed across northeastern, northern, central, and southwestern China, including Heilongjiang, Jilin, Liaoning, Hebei, Shandong, Henan, Hubei, Sichuan, and Yunnan.

Global distribution: Widely distributed in temperate and subtropical regions and is one of the most important cultivated edible mushrooms worldwide.

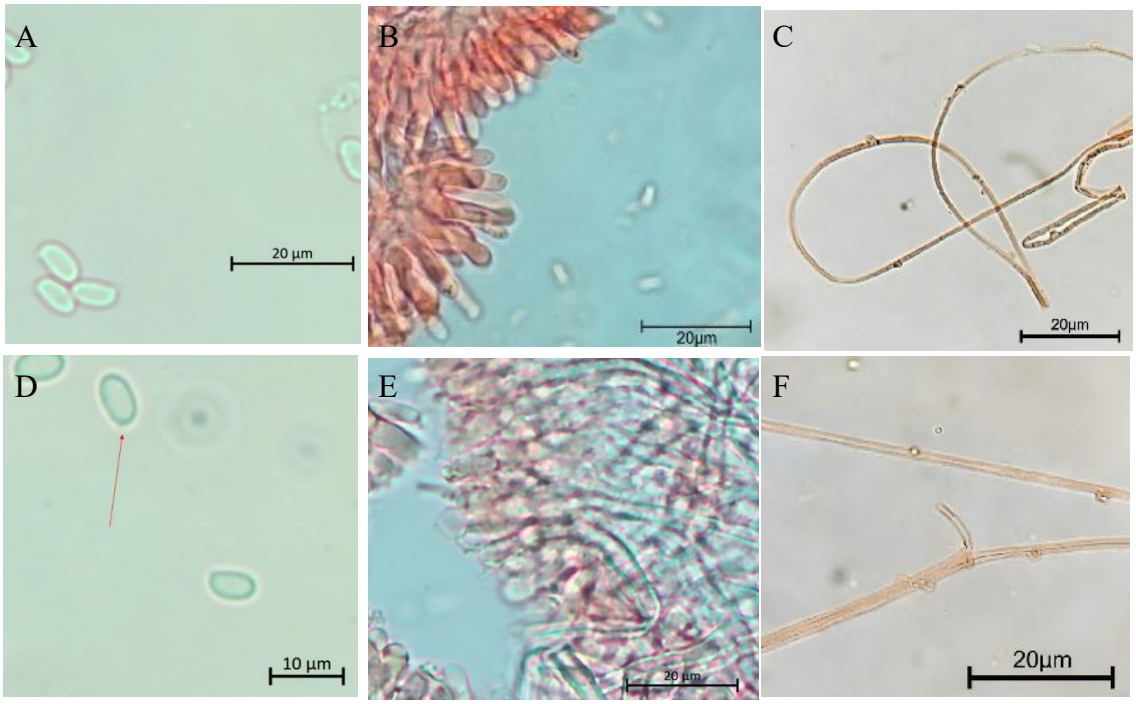

Figure 2.4 *Pleurotus ostreatus* (Jacq.) P. Kumm.
A: CP basidiospores; B: CP basidia; C: CP mycelium with clamp connections;
D: CPH basidiospores; E: CPH basidia; F: CPH mycelium with clamp connections.

2.3.2 Analysis of genetic relationships among strains

ITS-PCR amplification results: Genomic DNA from four parental strains was amplified using ITS region PCR. Agarose gel electrophoresis results (Fig. 2.5) showed clear single target bands without nonspecific amplification in all strains. The amplified fragment size was approximately 600–700 bp, indicating stable and reliable amplification suitable for subsequent sequencing analysis Schoch, C.L., et al. (2012). ITS sequences of each parental strain were subjected to BLAST analysis, and highly homologous reference sequences were selected to construct a phylogenetic tree (Fig. 2.5).

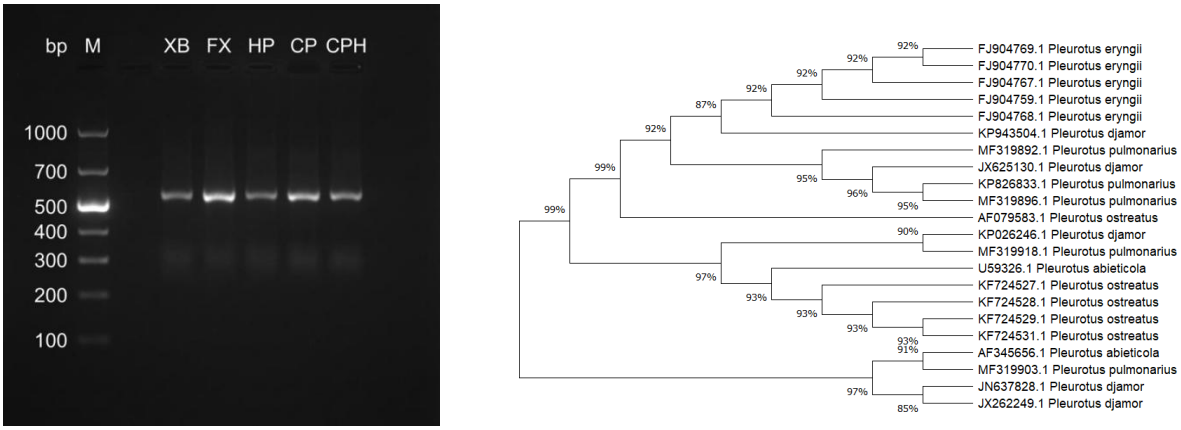

Figure 2.5 Phylogenetic analysis of parental genetic relationships

The results showed that the constructed phylogenetic tree had a high bootstrap support value of 99%, indicating strong confidence in the branching structure. The four parental strains were clearly divided into two major clades. *Pleurotus eryngii* formed an independent lineage and clustered closely with reference sequences from GenBank, showing a relatively large genetic distance from the other three parental strains. *Pleurotus djamor*, *Pleurotus ostreatus*, and *Pleurotus pulmonarius* were grouped into the second clade, indicating relatively close genetic relationships among them, although clear species-level genetic differentiation was still observed.

These results demonstrate that the tested parental strains have distinct genetic backgrounds and well-resolved phylogenetic relationships, providing a reliable molecular basis for subsequent interspecific hybrid breeding.

4. **Overall Conclusion**

This study provides a systematic and comprehensive evaluation of wild *Pleurotus* germplasm resources, focusing on their morphological characteristics, genetic relationships, physiological traits, and domestication potential. Through integrated analyses of morphology, ITS-based molecular identification, mycelial growth behavior, substrate utilization, and cultivation performance, a set of candidate parental strains with distinct biological characteristics was successfully identified. In addition, a

standardized workflow for monokaryotic isolation, interspecific hybridization, progeny screening, and hybrid authenticity verification was established, providing a solid methodological foundation for future breeding programs in *Pleurotus* species.

The results revealed substantial interspecific variation among the tested strains in both vegetative growth and reproductive performance. Phylogenetic analysis based on ITS sequences clearly separated the parental strains into distinct evolutionary clades, confirming their genetic divergence and supporting their suitability for distant hybridization studies. Physiological experiments further demonstrated that each strain exhibited specific preferences for carbon and nitrogen sources, indicating strong genotype-dependent metabolic regulation. These differences highlight the metabolic diversity within the genus *Pleurotus* and provide important guidance for optimizing culture conditions in industrial and breeding applications.

Cultivation experiments showed that mycelial growth rate, spawn run time, and yield performance were not always positively correlated. In particular, some strains with relatively slow vegetative growth demonstrated superior fruiting performance and higher biological efficiency, suggesting that reproductive potential is influenced by more complex physiological and genetic factors than simple mycelial expansion ability. This decoupling between vegetative growth and yield underscores the importance of multi-trait evaluation in strain selection and breeding strategies. In hybridization experiments, different interspecific combinations produced highly variable outcomes. Some combinations successfully generated stable hybrid progenies capable of completing the full life cycle and producing normal fruiting bodies, indicating partial compatibility between parental genomes. In contrast, other combinations exhibited developmental arrest at early stages or complete incompatibility, reflecting strong reproductive barriers and genetic distance between certain species. These findings confirm that interspecific hybridization in *Pleurotus* is highly genotype-dependent and requires careful parental selection.

Importantly, transcriptomic analysis of the successfully obtained H–FX hybrid provided molecular-level evidence supporting the observed phenotypic traits. The results revealed a global gene expression pattern biased toward parent FX, suggesting a dominant parental regulatory influence during hybrid development. This expression bias may be associated with regulatory dominance, allele-specific expression, or epigenetic inheritance mechanisms. Such molecular evidence not only explains the phenotypic tendencies observed in the hybrid but also provides new insights into gene expression regulation in fungal hybrids. Overall, this study demonstrates the feasibility of utilizing wild *Pleurotus* germplasm as valuable genetic resources for breeding improvement. It highlights the potential of interspecific hybridization as an effective strategy for generating novel strains with desirable agronomic traits, while also emphasizing the biological limitations imposed by reproductive compatibility barriers. The integration of morphological, physiological, molecular, and transcriptomic approaches provides a robust framework for future studies on fungal breeding and functional genomics.

In conclusion, the findings of this research contribute to the advancement of *Pleurotus* breeding strategies by identifying elite parental resources, elucidating interspecific compatibility patterns, and revealing gene expression dynamics in hybrid progenies. These results not only support the development of improved edible mushroom cultivars but also provide a theoretical basis for understanding evolutionary relationships and regulatory mechanisms in basidiomycete fungi. Further studies focusing on multi-generation stability, genome-wide interaction analysis, and environmental adaptability will be essential to fully exploit the breeding potential of wild *Pleurotus* resources.

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Author Contributions

W.W: Conceptualization, methodology, software, writing—original draft; X.D: methodology, software, writing—review and editing; Q.W: supervision, writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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Data availability

All data generated or analyzed during this study are included in this article. The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Consent for publication

Not Applicable

Conflicts of Interest

The authors declare no conflict of interest

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