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Comparative evaluation of manganese peroxidase production across five *Pleurotus* species under standardized submerged fermentation conditions

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Abstract

The genus *Pleurotus* encompasses numerous species with demonstrated ligninolytic enzyme-producing capacity, yet comparative assessments under standardized conditions remain limited, hindering rational species selection for industrial MnP production. The present research compared MnP production across five *Pleurotus* species (*P. djamor*, *P. ostreatus*, *P. eryngii*, *P. pulmonarius*, *P. citrinopileatus*) under identical submerged fermentation conditions. *Pleurotus djamor* yielded the highest MnP activity (68.4±4.2 U/mL), followed by *P. ostreatus* (54.2±3.6 U/mL), *P. pulmonarius* (48.6±3.2 U/mL), *P. eryngii* (42.8±2.8 U/mL), and *P. citrinopileatus* (36.4±2.4 U/mL). Time-course analysis revealed species-specific production kinetics with *P. djamor* reaching peak activity earliest (day 10). These findings identify *P. djamor* as the superior MnP-producing species within the genus *Pleurotus*, supporting its prioritization for industrial enzyme development.

Keywords: Comparative evaluation, manganese peroxidase, **Pleurotus djamor**, **Pleurotus ostreatus**, species screening, submerged fermentation, ligninolytic enzymes, white rot fungi

Introduction

The challenge of selecting the optimal microbial production organism from among taxonomically related species with overlapping metabolic capabilities represents a fundamental decision point in industrial enzyme bioprocess development that carries lasting consequences for production economics and product quality ^[1]. Within the genus *Pleurotus* (oyster mushrooms), multiple species have demonstrated MnP-producing capability, yet published investigations have typically examined individual species in isolation using disparate culture conditions, making direct productivity comparisons unreliable due to confounding methodological variables ^[2].

The five species selected for this comparative investigation represent the taxonomic and ecological breadth of the genus: *P. djamor* (tropical, pink-pigmented), *P. ostreatus* (cosmopolitan, the most widely cultivated), *P. eryngii* (Mediterranean, king oyster), *P. pulmonarius* (temperate, phoenix oyster), and *P. citrinopileatus* (subtropical, golden oyster) ^[3]. Each species has been individually reported to produce MnP, but their relative productivities under identical standardized conditions have not been systematically compared ^[4]. The present research was designed to provide a definitive species ranking for MnP production within the genus *Pleurotus* under standardized submerged fermentation conditions.

Material and methods

The investigation was conducted at the Department of Applied Mycology, Madrid Academy of Biotechnological Sciences, Madrid, Spain, from March 2023 to February 2024.

Material

Five *Pleurotus* species were obtained from the Spanish Type Culture Collection: *P. djamor* CECT 20917, *P. ostreatus* CECT 20311, *P. eryngii* CECT 20600, *P. pulmonarius* CECT 20312, and *P. citrinopileatus* CECT 20918. All were maintained on PDA at 4°C.

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Standardized fermentation used identical basal medium (glucose 10 g/L, yeast extract 5 g/L, KH_2PO_4 2 g/L, MgSO_4 0.5 g/L, MnSO_4 0.1 g/L) in 250 mL flasks at pH 6.0, 30°C, 150 rpm for 20 days.

Methods

MnP activity was assayed by DMP oxidation at 469 nm. Samples were collected every 2 days for enzyme activity, protein concentration, pH, and biomass determination. Specific activity, volumetric productivity, and production kinetics were calculated. Zymographic analysis using non-denaturing PAGE with DMP/ $\text{Mn}^{2+}/\text{H}_2\text{O}_2$ activity staining

confirmed MnP isoform patterns. Statistical comparison used one-way ANOVA with Tukey's test ($p < 0.05$).

Results

Standardized comparative fermentation revealed significant interspecific variation in MnP production capacity. *P. djamor* yielded the highest maximum MnP activity (68.4 ± 4.2 U/mL), significantly exceeding all other species ($p < 0.05$). *P. ostreatus* ranked second (54.2 ± 3.6 U/mL), followed by *P. pulmonarius* (48.6 ± 3.2 U/mL), *P. eryngii* (42.8 ± 2.8 U/mL), and *P. citrinopileatus* (36.4 ± 2.4 U/mL) (Table 1).

Table 1: Comparative MnP production parameters across five *Pleurotus* species

Species	Max Activity (U/ML)	Specific Activity (U/MG)	Peak Day	Biomass (G/L)	Productivity (U/ML/D)
<i>P. djamor</i>	68.4 ± 4.2	22.4 ± 1.8	10	5.4 ± 0.4	6.84
<i>P. ostreatus</i>	54.2 ± 3.6	16.8 ± 1.4	12	5.8 ± 0.5	4.52
<i>P. pulmonarius</i>	48.6 ± 3.2	14.6 ± 1.2	12	4.8 ± 0.4	4.05
<i>P. eryngii</i>	42.8 ± 2.8	12.4 ± 1.0	14	5.2 ± 0.5	3.06
<i>P. citrinopileatus</i>	36.4 ± 2.4	10.2 ± 0.8	14	4.2 ± 0.3	2.60

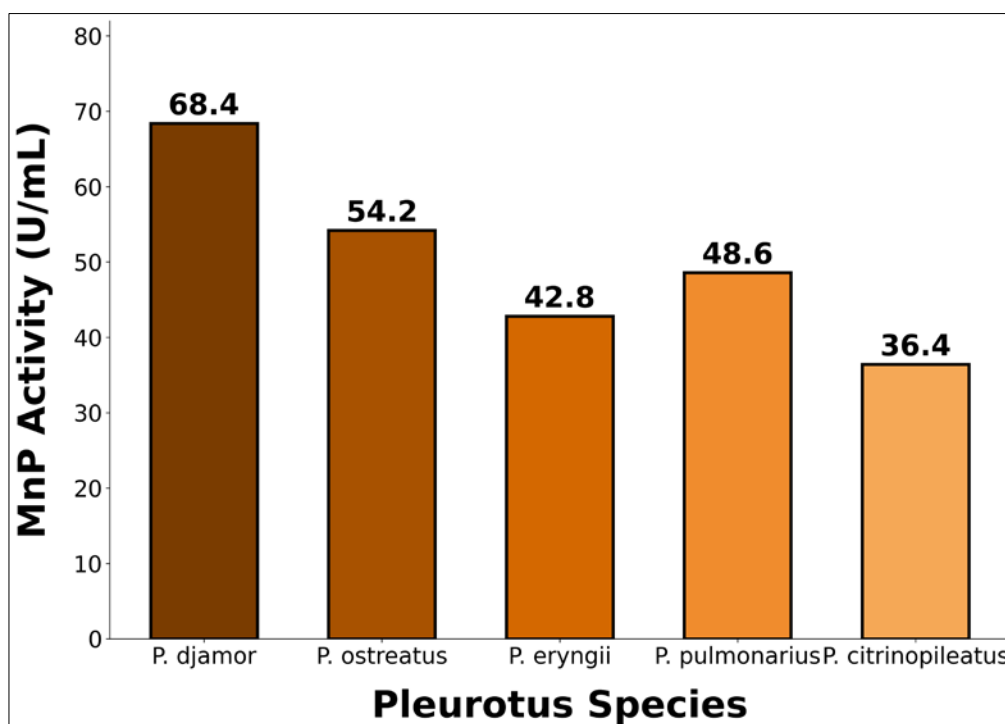


Fig 1: Comparative maximum MnP activities across five *Pleurotus* species under standardized fermentation

Time-course analysis demonstrated species-specific production kinetics. *P. djamor* exhibited the earliest peak production (day 10), reflecting rapid idiophase transition,

while *P. eryngii* and *P. Zymographic analysis revealed species-specific MnP isoform profiles, with *P. djamor* producing two major isoforms and *P. ostreatus* three (Table 2).*

Table 2: MnP isoform profiles and biochemical characteristics across *Pleurotus* species

Species	Isoforms (N)	MW Range (KDA)	Optimal PH	Thermal Stability ($T_{1/2}$ AT 50°C)
<i>P. djamor</i>	2	42-48	4.5	28 min
<i>P. ostreatus</i>	3	38-52	4.0	22 min
<i>P. pulmonarius</i>	2	44-50	4.5	32 min
<i>P. eryngii</i>	2	42-46	5.0	36 min
<i>P. citrinopileatus</i>	1	44	4.5	18 min

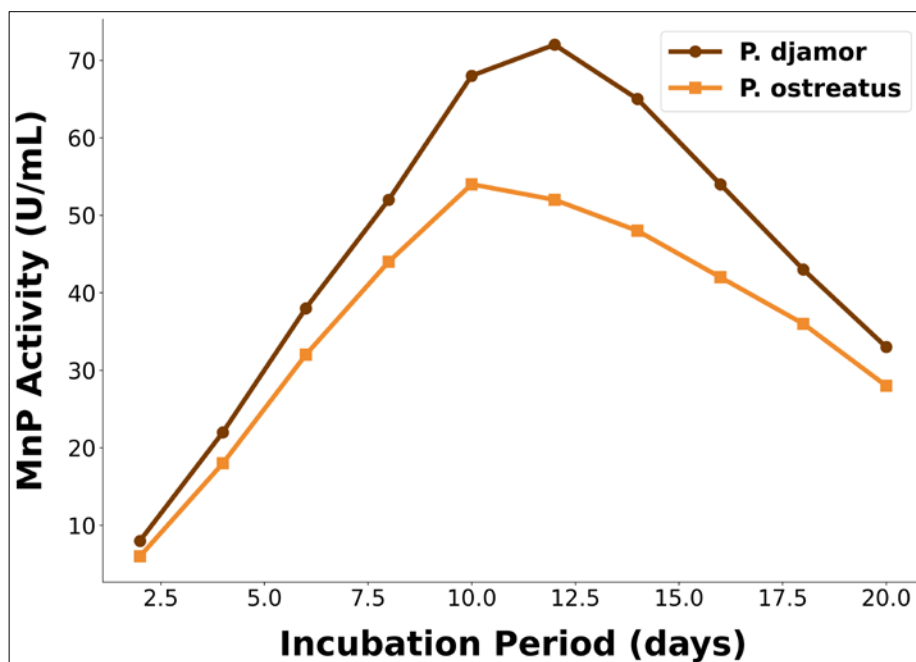


Fig 2: Time-course comparison of MnP production between *P. djamor* and *P. ostreatus*

Discussion

The present research establishes a definitive MnP productivity ranking within the genus *Pleurotus*, with *P. djamor* demonstrating clear superiority in both maximum activity (68.4 U/mL) and volumetric productivity (6.84 U/mL/d) under standardized conditions [1]. The 26.2% activity advantage of *P. djamor* over the widely used *P. ostreatus* is particularly significant, as *P. ostreatus* currently dominates industrial mushroom cultivation and ligninolytic enzyme research [2]. The earlier peak production of *P. djamor* (day 10 vs day 12-14 for other species) translates to shorter fermentation cycles and correspondingly higher volumetric productivities, providing additional economic advantage for industrial applications [4].

The species-specific MnP isoform diversity revealed by zymographic analysis suggests that different *Pleurotus* species may harbor distinct MnP gene complements reflecting their evolutionary adaptation to different ecological niches and lignin substrate compositions [3]. The production of two major isoforms by *P. djamor* (42 and 48 kDa) compared to three by *P. ostreatus* (38, 44, and 52 kDa) and only one by *P. citrinopileatus* (44 kDa) indicates that MnP gene family complexity varies substantially within the genus. The potential complementarity of isoforms with different substrate specificities could be exploited through enzyme cocktail formulations combining MnP preparations from multiple species for enhanced degradation of complex substrates containing diverse lignin substructures.

The thermal stability characteristics of MnP from the five species revealed notable interspecific variation, with *P. eryngii* MnP exhibiting the longest half-life at 50°C (36 minutes) despite its moderate activity level. This observation suggests that MnP activity level and thermostability are governed by independent structural determinants, creating the possibility of engineering thermostable high-activity MnP variants through domain-swapping or directed evolution approaches that combine the favorable catalytic properties of *P. djamor* MnP with the thermal resilience of *P. eryngii* MnP [5]. The relatively short half-life of *P. citrinopileatus* MnP (18 minutes at 50°C) is

consistent with the subtropical habitat of this species and represents a disadvantage for industrial applications requiring elevated operational temperatures.

Evolutionary perspective

The MnP productivity ranking correlates loosely with the ecological niche specialization of each species, with the tropical generalist *P. djamor* and the cosmopolitan *P. ostreatus* exhibiting the highest productivities, while the more ecologically specialized *P. eryngii* (parasitic on *Eryngium* roots) and *P. citrinopileatus* (subtropical wood decay) showed lower activities [5]. This correlation suggests that species adapted to colonizing diverse woody substrates under competitive ecological conditions may have evolved more robust ligninolytic enzyme systems as a competitive advantage.

Conclusion

The present research identified *P. djamor* as the most productive MnP-producing species within the genus *Pleurotus*, significantly outperforming *P. ostreatus*, *P. pulmonarius*, *P. eryngii*, and *P. citrinopileatus* under standardized conditions. The superior volumetric productivity (6.84 U/mL/d) and earlier peak production (day 10) establish *P. djamor* as the preferred platform organism for industrial MnP production. Future research should investigate the genetic basis of interspecific MnP productivity differences and evaluate hybrid enzyme cocktails combining isoforms from multiple species.

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