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Evaluation of mycelial growth of *Pleurotus spp.* in a potato infusion-based culture medium (*Solanum tuberosum*)

Evaluación del crecimiento micelial de *Pleurotus spp*. en un medio de cultivo a base de infusión de papa (*Solanum tuberosum*)

Sarahi Priscilla Ramírez Domínguez[a], *Paul Misael Garza López*[a], *Gabriela de Vega Luttmann*[a], *Sergio Hernández León*[a], *Carlos Farfán Flores*[a], *Oscar Arce Cervantes*[a*]

Abstract:

The in vitro mycelial growth rate of 3 *Pleurotus* strains (*P. agaves, P. djamor,* and *P. ostreatus*) was studied in two culture media: PDA 39g/L and potato infusion culture medium. Mycelial growth was measured for 7 days, and the growth curve was plotted. The results were analyzed using a completely randomized design. Statistical analysis was performed under ANOVA, followed by a Tukey test. The *P. djamor* strain grown on potato infusion medium presented the highest growth rate, with significant differences ($p < 0.05$) compared to the other treatments. P. ostreatus showed high and constant growth in both media, without significant differences. P. agaves showed the lowest growth, without a significant response to the medium used. The potato infusion-based culture medium represents a highly viable alternative for the in vitro growth of edible fungi, due to its low cost, ease of preparation, and biological effectiveness.

Keywords:

Pleurotus, culture media, in vitro, growth rate, mycelial

Resumen:

Se estudió la velocidad de crecimiento micelial in vitro de 3 cepas de *Pleurotus* (*P. agaves, P. djamor* y *P. ostreatus*) en 2 medios de cultivo: PDA 39 g/L y medio de cultivo a base de infusión de papa. Midiendo el crecimiento micelial durante 7 días y se trazó la curva de crecimiento. Los resultados fueron analizados con un diseño completamente al azar; el análisis estadístico se realizó bajo un ANOVA, y posteriormente se aplicó una prueba de Tukey. La cepa de *P. djamor* cultivada en el medio de infusión de papa presentó la mayor tasa de crecimiento, con diferencias significativas ($p < 0.05$) respecto a los demás tratamientos. P. *ostreatus* mostró un crecimiento elevado y constante en ambos medios, sin diferencias significativas. *P. agaves* mostró el menor crecimiento, sin respuesta significativa al medio utilizado. El medio de cultivo a base de infusión de papa representa una alternativa altamente viable para el crecimiento in vitro de hongos comestibles, debido a su bajo costo, facilidad de preparación y efectividad biológica.

Palabras Clave:

Pleurotus, medios de cultivo, in vitro, velocidad de crecimiento, micelial

[a] Instituto de Ciencias Agropecuarias. Universidad Autónoma del Estado de Hidalgo. Sarahi Priscilla Ramírez-Domínguez, https://orcid.org/0009-0008-7528-8236, ra357704@uaeh.edu.mx; Paul Misael Garza-López, https://orcid.org/0000-0002-3151-8369, paul_garza@uaeh.edu.mx; Gabriela De Vega-Luttmann, https://orcid.org/0000-0002-5745-739X, gabriela_devega@uaeh.edu.mx; Sergio Hernández-León, https://orcid.org/0000-0001-5892-6066, sergio_hernandez@uaeh.edu.mx; Carlos Farfán-Flores, https://orcid.org/0009-0006-8117-7351, fa383820@uaeh.edu.mx; Oscar Arce-Cervantes, https://orcid.org/0000-0002-3388-2973, oarce@uaeh.edu.mx.

* Autor de Correspondencia: Email: oarce@uaeh.edu.mx.

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1. Introduction

In biology, one of the most interesting groups is fungi, due to their diversity and abundance. They play a crucial ecological role in ecosystems by acting as decomposers of organic matter. It is estimated that there are 1,500,000 fungal species worldwide, of which only 5% are known. Of these, only about 2,000 species are edible, and just 36 are cultivated commercially and industrially [1].

The global macrofungi industry consists primarily of three categories: wild mushrooms, cultivated edible mushrooms, and mushrooms with medicinal properties. In 2013, this industry was valued at $63 billion, with cultivated edible mushrooms accounting for 54% of the total value, medicinal mushrooms for 38%, and wild mushrooms for 8%. Five genera make up roughly 85% of global production: *Lentinula edodes* (shiitake) with 22%, *Pleurotus spp*. 19%, *Auricularia spp*. 18%, *Agaricus bisporus* 15%, *Flamulina spp*. 11%, *Volvarella spp*. 5%, and other species 10% [6].

Global production of *Pleurotus* spp. is concentrated in Asia, with China as the leading producer, 87% of the world's total. Japan, South Korea, Taiwan, Thailand, Vietnam, and India, which together contribute 12%, while Europe and the Americas make up the remaining 1%. According to Sánchez and Royse [12], edible mushroom consumption reached 4.78 kg per person per year (13.7 g daily). The empirical cultivation of *Pleurotus* species began in Germany around 1917, using wild mycelium to inoculate logs. However, the first large-scale cultivation was not achieved until 1969 in Hungary.

In Mexico, the production of mushrooms from the genus *Pleurotus* began in 1974 in the municipality of Cuajimalpa. The most significant mushroom production in the country is in the states of Chiapas, Guanajuato, Jalisco, Mexico City, Morelos, Puebla, and Veracruz. In 2014, production reached 3,000 metric tons, representing a 39% increase and positioning the country as the 3rd largest producer in the Americas [9]. Since then, both small- and large-scale cultivation have expanded rapidly across various regions of the world, utilizing locally available agricultural byproducts, agro-industrial waste, and forestry residues for production. Globally, the button mushroom (*Agaricus*) ranks first in production volume, followed by *Pleurotus spp*. and *Lentinula edodes* (shiitake).

The boom in commercial production of *Pleurotus spp*. can be attributed to several factors: 1) the existence of numerous potentially cultivable |species; 2) simple and low-cost production technologies; 3) the development of strains adapted to different temperatures and substrates; and 4) the evolution of cultivation methods, which have progressed from artisanal techniques to highly technological processes [12].

However, the primary culture media used for strain preservation and propagation include Potato Dextrose Agar, Malt Extract Agar, and Sabouraud Dextrose Agar, which are highly recommended for fungal growth. Nevertheless, the use of these commercial culture media represents a significant cost for small- and medium-scale production systems. The use of potato infusion as a significant component in culture media, such as potato dextrose agar, has become standard practice in the research and production of edible fungi. Potato (*Solanum tuberosum*) provides a rich source of carbohydrates, mainly starch, as well as essential vitamins and minerals that promote mycelial growth under in vitro conditions. These nutrients allow the initial development of mycelium with adequate morphological characteristics. In edible species such as *Pleurotus ostreatus*, *Lentinula edodes,* and *Ganoderma lucidum*, PDA medium has proven to be efficient to evaluate the viability and growth rate of different strains, perform compatibility tests, and standardize culture conditions [2]. Its simple preparation, low cost, and wide availability make it ideal for laboratory applications as well as for artisanal and commercial production systems. For these reasons, potato-based media continue to be a fundamental tool in the biotechnology of edible fungi, facilitating physiological, genetic, and ecological research, as well as the improvement of production processes [10].

The potato infusion-based culture medium allows an efficient mycelial growth of edible fungi comparable or superior to that obtained in traditional culture

media, representing a viable and lower-cost alternative for use in laboratories and by local producers.

Therefore, the objective of this project is to evaluate the use of potato (*Solanum tuberosum*) infusion culture medium on the mycelial growth rate of three *Pleurotus spp*—fungal strains.

2. Materials and methods

Acquisition and preservation of strains

Two strains were isolated from the spawn inoculum of *Pleurotus ostreatus* and *Pleurotus djamor*, which were donated by the Edible Mushroom Biotechnology Laboratory at Universidad Autónoma Metropolitana Iztapalapa. The *Pleurotus agaves* strain was obtained from the strain collection of the Institute of Agricultural Sciences at Universidad Autónoma del Estado de Hidalgo.

For strain isolation and preservation, three culture media were used: PDA (Potato Dextrose Agar) prepared at 39 g/L, SDA (Sabouraud Dextrose Agar) at 65 g/L, and MEA (Malt Extract Agar) at 33.6 g/L. The media were sterilized in Erlenmeyer flasks using an autoclave for 15 minutes at 15 psi and 121°C.

Following this procedure, the media were poured into Petri dishes and allowed to solidify. They were then inoculated with a mycelial explant developed on the spawn and maintained at temperatures of 21 ± 2°C for 5 to 10 days to promote fungal growth. Subsequently, the Petri dishes were placed in refrigeration [7].

Mycelial Growth Rate

The radial growth rate on plates was evaluated for *P. ostreatus*, *P. djamor*, and *P. agaves*. Five replicates were used per treatment, totaling 30 observations (Table 1). Using PDA culture media and the proposed potato infusion-based medium: 1) PDA (39 g/L), 2) Agar with dextrose and agar: 250 g of potato (*Solanum tuberosum*) were cut into squares and boiled in 500 mL of distilled or purified water for 10-15 min to obtain the solution that will be used for the culture medium [5]. The potato extract was filtered and adjusted to a final volume of 1 L. Then, 15 g/L of agar was added, and the mixture was heated on a constant stirring rack for 2 min until the culture medium was homogenized.

The two-culture media were sterilized in Erlenmeyer flasks using an autoclave at 15 psi and 121°C for 15 minutes.

Subsequently, they were poured into 90 mm diameter Petri dishes and allowed to solidify. An actively growing mycelial plug (~1 cm²) was inoculated at the center of each Petri dish. The dishes were sealed with Parafilm and labeled with the corresponding strain designation. Two perpendicular axes were drawn on the base of each plate to measure radial growth. Mycelial expansion was recorded every 24 hours along these axes until complete plate colonization was achieved. The experiment ran for 7 days under complete darkness at 27°C [7].

Strain	Treatment	Repetitions
P. djamor	PDA	5
	Potato	5
P. ostreatus	PDA	5
	Potato	5
P. agaves	PDA	5
	Potato	5

Table 1. *Total number of repetitions per treatment*

Adjustment of mycelial growth kinetics.

The mycelial growth data were fitted to the modified Gompertz model [8], which describes sigmoidal growth using the following equation:

$$y(t) = X_{max} * exp\ exp\left(-exp\left(\frac{\mu_{max} * e}{A}(\lambda - t) + 1\right)\right)$$

Where:

X_{max} = The maximum (asymptotic) growth value

μ_{max} = The maximum specific growth rate

λ = the adaptation time or “lag phase”.

A = is the horizontal asymptote, representing the maximum or stable value that the system can reach.

The data were fitted using R software in the RStudio environment (version 4.4.2). Using a nonlinear model (Levenberg-Marquardt), initial values were set for each parameter based on the experimental data: the maximum observed value for Xmax, an approximate value of 0.1 for μmax, and the mean of the recorded time for λ.

Statistical Analysis

A completely randomized design with a 3 × 2 factorial arrangement was implemented. The data were analyzed using two-way ANOVA for treatment and *Pleurotus* strain effects at a 95% confidence

level, followed by Tukey's test. All analyses were performed using RStudio software (version 4.4.2).

3. Results and Discussion

The following section presents the findings from the mycelial growth evaluation.

The mycelial growth rate (mm/day) of three *Pleurotus* strains (*P. agaves*, *P. djamor*, and *P. ostreatus*) was evaluated on two culture media (PDA and potato infusion). The results show significant differences between treatments according to Tukey's test ($p < 0.05$) (Figure 1).

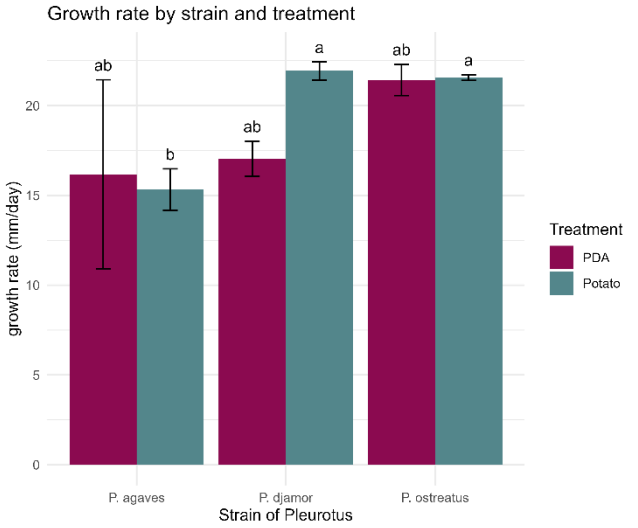

Figure 1. *Mycelial growth rate*

The *P. djamor* strain (group a) grown on potato infusion medium showed the highest mycelial growth rate (21.92 ± 0.41 mm/d), with statistically significant differences compared to PDA (17.03 ± 0.79 mm/d). While for *P. ostreatus* no significant differences were observed between potato infused medium and PDA medium (21.42 ± 0.7 mm/d and 21.55 ± 0.13 mm/d respectively) On the other hand, *P. agaves* strain (group c) on potato infused medium showed the lowest growth rate (15.32 ± 0.94 mm/d), significantly lower than *P. djamor* on potato and *P. ostreatus* on both media.

For *P. ostreatus*, no significant differences were observed between the culture media, indicating stable performance across different substrates. In the case of *P. agaves*, growth was slightly higher on PDA than on the potato medium, though this difference was not statistically significant. These results demonstrate that the culture medium significantly influences mycelial growth, depending on the strain used. Notably, *P. djamor* showed a positive response to the potato infusion medium, while *P. Ostreatus* maintained robust development regardless of the treatment.

This behavior aligns with findings reported by Sánchez [13], who describes *P. djamor* as a highly adaptive strain with a rapid growth rate in media rich in readily assimilable carbon.

The results were fitted to the Gompertz model (Figure 2). The *P. ostreatus* strain exhibited the highest mycelial growth, reaching an area of approximately 36 mm by day 7. This strain displayed a short adaptation phase, followed by a well-defined logarithmic phase between days 3 and 6, and a stationary phase at the end of the period. These results align with those of De Vega Luttmann [4], where an initial latency phase was observed, followed by exponential growth and finally a phase parallel to the horizontal axis. *P. djamor* showed good mycelial development, though slightly lower than *P. ostreatus.*

P. agaves showed the slowest growth, with a final mycelial area of 31 mm. This strain exhibited a more extended adaptation phase and more linear growth, without as pronounced a logarithmic phase as the other strains.

These results demonstrate significant differences in colonization capacity among the evaluated strains, with *P. ostreatus* being the most efficient in terms of both mycelial growth rate and expansion under the applied treatment conditions.

These results are similar to those reported by Coello-Loor et al. [3], who found that Pleurotus ostreatus exhibited higher radial growth rates on two culture media (PDA and rice husk + PDA), although significantly lower than those obtained in the present study.

After 4 days of cultivation, all three strains showed similar growth of approximately 25.0 mm, with no statistical differences (p-value > 0.5). However, after 24 hours, *Pleurotus djamor* and *Pleurotus ostreatus* exhibited greater increase in the potato infusion medium. From day 5 onward, significant differences in mycelial area were observed (Table 2) with 50% greater mycelial area compared to *P. ostreatus* and *P. djamor* strain.

Zamora et al. [9] evaluated radial growth kinetics for mycelium production in *Pleurotus ostreatus*, using a potato infusion-based culture medium supplemented with young corn cob husks and

legume shells (pea, bean, and fava bean), reporting results similar to those presented in this study.

The potato infusion culture medium has proven to be highly efficient for the cultivation of edible fungi under laboratory conditions. Compared to traditional and synthetic culture media, potato infusion medium has significant advantages from both biological and economic points of view.

First, the natural composition of potato extract provides a complex mixture of nutrients including carbohydrates (mainly starch), soluble sugars, minerals, and B-complex vitamins, essential elements for optimal mycelial development [13]. Unlike synthetic media, whose chemical formulation is usually precise but limited to certain defined compounds, this culture medium offers nutrition closer to what fungi find in their natural substrates.

In addition, the simplicity in its preparation and the local availability of ingredients make this medium an accessible option for small producers, research laboratories, and teaching processes. Compared to commercial media that require expensive inputs or difficult access, potato infusion medium can be prepared with basic materials without compromising the quality of the culture.

The results obtained in this study show that *Pleurotus djamor* shows a significantly higher mycelial growth in potato infusion medium compared to commercial PDA. This positive response can be explained by several biochemical factors related to the nutritional composition of the medium.

Potato, used as the base of the culture medium, is rich in starch, a polysaccharide that amylolytic enzymes produced by P. djamor can degrade. This enzymatic capacity allows the fungus to obtain a sustained source of carbon from the hydrolysis of starch into simpler sugars, thus facilitating steady and vigorous mycelial growth [13]. In addition, during potato cooking, soluble sugars such as glucose and maltose are released, which are easily assimilated by the fungus, favoring rapid colonization of the medium from the early stages of cultivation.

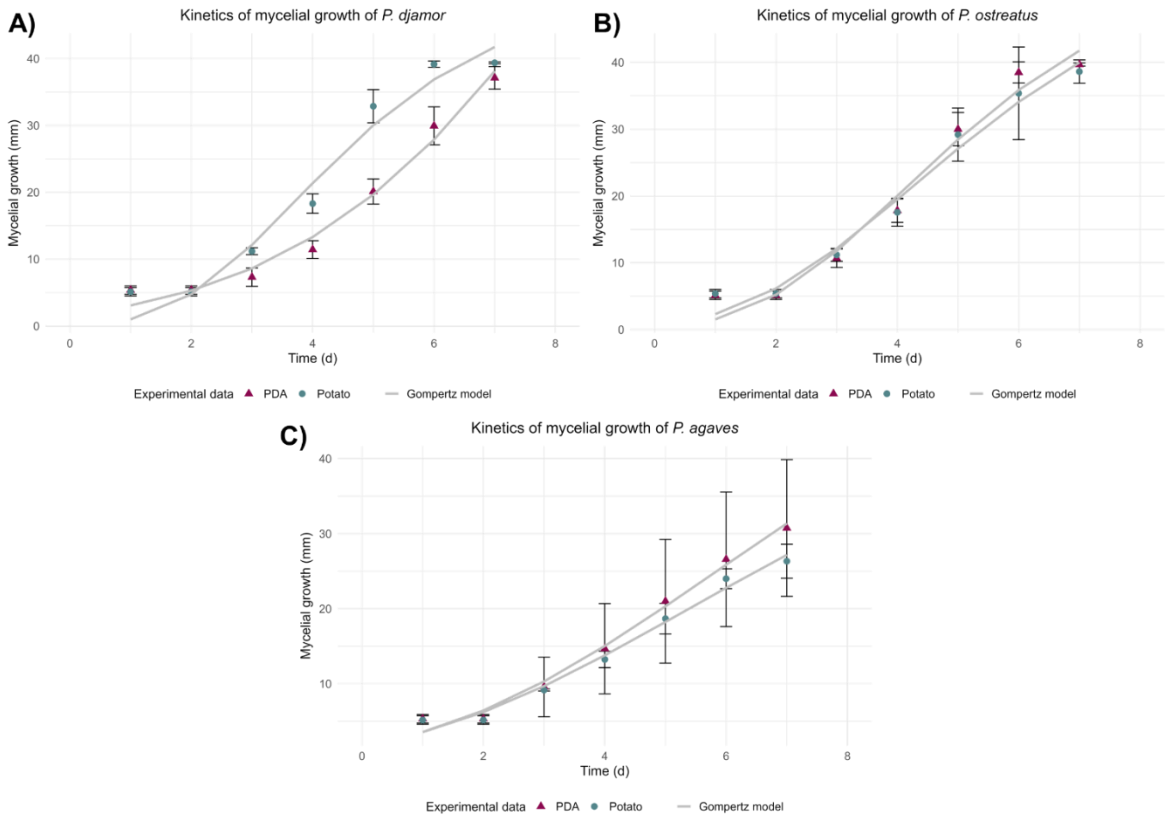

Figure 2. Kinetics of mycelial growth of *Pleurotus* adjusted to the Gompertz model; A) *P.djamor*, B) *P. ostreatus,* and C) *P. agave*

Table 2. Parameters of the Gompertz model fitted to the growth kinetics of *Pleurotus spp.*

Strain	Treatment	A	μ_{max}	λ	R^2
P. djamor	PDA	56.51 ± 8.49	8.68 ± 0.27	1.68 ± 0.18	0.981 ± 0.001
	Potato	51.09 ± 4.97	9.55 ± 0.96	1.74 ± 0.15	0.966 ± 0.007
P. ostreatus	PDA	57.52 ± 8.58	8.75 ± 0.32	1.71 ± 0.21	0.975 ± 0.005
	Potato	73.52 ± 36.54	8.34 ± 0.32	1.79 ± 0.57	0.973 ± 0.004
P. agaves	PDA	58.52 ± 8.45	8.66 ± 0.32	1.65 ± 0.19	0.975 ± 0.005
	Potato	54.16 ± 6.11	4.56 ± 0.29	1.00 ± 0.14	0.972 ± 0.014

4. Conclusions

The results obtained demonstrate that potato infusion medium not only represents a viable alternative to traditional culture media but also positions itself as an efficient and functional option for species of edible fungi, particularly *Pleurotus djamor*. This strain showed faster, more uniform mycelial growth, suggesting that the biochemical composition of the medium is optimally adjusted to its metabolic requirements.

Importantly, unlike more expensive or technically demanding media, potato infusion medium can be prepared simply and inexpensively without compromising culture quality. This combination of affordability and efficient biological performance positions it as a strategic tool for research, strain preservation, and initial fungal production.

The use of potato infusion culture media represents a viable alternative for small-to-medium macromycete producers, serving as an economical and readily accessible medium for fungal mycelium conservation and propagation. This study highlights the importance of optimizing growth conditions in the proposed culture medium.

Conflicts of interest

The authors declare they have no conflicts of interest.

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