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Original article

Search for new naturally occurring strains of *Pleurotus* to improve yields. *Pleurotus albidus* as a novel proposed species for mushroom production

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ABSTRACT

Background: The species of genus *Pleurotus* are worldwide cultivated.
Aims: To evaluate growth, yield production and morphological variations of fruiting bodies obtained from the cultivation of fourteen naturally occurring *Pleurotus* strains isolated from Argentina.
Methods: The strains growth was tested at different temperatures on Nobles' medium. Substrates assayed were: supplemented *Salix* sawdust, supplemented and non supplemented wheat straw. The species studied were *Pleurotus albidus*, *Pleurotus cystidiosus*, *Pleurotus djamor*, *Pleurotus ostreatus* and *Pleurotus pulmonarius*.
Results: The maximum rate growth was reached by strains of *P. pulmonarius*, *P. albidus*, and *P. ostreatus*. No relationship was found when optimal mycelium growth, incubation time and yields were compared. The highest yield was obtained with *P. albidus* on wheat straw (biological efficiency 171.3%) which overcame in 82% the yield obtained for the commercial strain in the same substrate. When morphological variations were analyzed for each species, significant differences were found among strains. It was also possible to find a naturally occurring strain of *P. ostreatus* with better biological efficiency than the commercial strain assayed.
Conclusions: We propose the study of naturally occurring strains as a useful practice to improve yields of species of *Pleurotus*. Due to the high biological efficiency obtained we propose *P. albidus* as a new species for commercial production.

Mejora de los rendimientos en la producción de *Pleurotus* mediante la utilización de nuevas cepas silvestres. *Pleurotus albidus*, una nueva especie propuesta para la producción de hongos comestibles

Palabras clave:
Pleurotus albidus
Pleurotus cystidiosus
Pleurotus djamor
Pleurotus ostreatus
Pleurotus pulmonarius
Cultivo de hongos comestibles
Desechos lignocelulósicos
Cepas silvestres

RESUMEN

Antecedentes: Las especies del género *Pleurotus* son cultivadas en todo el mundo.
Objetivos: Evaluar el crecimiento, la producción y la variación morfológica de las fructificaciones obtenidas en cultivo de catorce cepas silvestres de *Pleurotus* aisladas en la Argentina.
Métodos: Las cepas fueron evaluadas a diferentes temperaturas de crecimiento en medio de Nobles. Se emplearon como sustrato aserrín de *Salix* suplementado, paja de trigo suplementada y no suplementada. Las especies estudiadas fueron *Pleurotus albidus*, *Pleurotus cystidiosus*, *Pleurotus djamor*, *Pleurotus ostreatus* y *Pleurotus pulmonarius*.
Resultados: La máxima tasa de crecimiento fue lograda por las cepas de *P. pulmonarius*, *P. albidus* y *P. ostreatus*. No se encontraron relaciones entre el crecimiento micelial óptimo, el tiempo de incubación y los rendimientos. El mayor rendimiento se obtuvo con *P. albidus* en paja de trigo (eficiencia biológica 171,3%), que superó en un 82% el obtenido por la cepa comercial en el mismo sustrato. Al analizar las variaciones morfológicas para cada especie, se encontraron diferencias significativas entre las cepas. Fue posible encontrar una cepa silvestre de *P. ostreatus* con rendimientos superiores a la cepa control empleada.

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Conclusiones: Proponemos estudiar las cepas silvestres como práctica útil para incrementar los rendimientos en cultivo de las especies de *Pleurotus*. Por los altos rendimientos obtenidos, se propone a *P. albidus* como una nueva especie cultivable a nivel comercial.

The genus *Pleurotus* is very versatile, with a large number of edible species that grow in different environment conditions and substrates. The species of genus *Pleurotus* are relatively easy to produce on agriculture waste; this facilitates the development of mushroom farms which can produce at low prices in different geographic regions.

Several species of *Pleurotus* can be cultivated on trunks or formulated substrates. The culture on stumps and trunks is in use since the beginning of the 20th century and was clearly described by Falck[9] and Passecker[18]. An important innovation in the cultivation of *Pleurotus* species, developed by Block et al[4,5], was the use of sawdust. Eger[8] developed the culture of *Pleurotus* on corn waste. Massive production on straw was made by Herzig et al[10]; industrial production of substrate for fruiting was later developed[11,12,23,26,27,29]. Several researchers have focused on searching for new substrates for the oyster mushrooms cultivation, such as the utilization of paddy straw, maize stover, sugarcane bagasse, coir pith[19], leaves of hazelnut, leaves of *Tilia*, leaves of aspen[25], sunflowers seed hulls[6] and coffee pulp[25] among others.

In Argentina there are, so far, six species of *Pleurotus* found in nature, namely *Pleurotus albidus*, *Pleurotus cystidiosus*, *Pleurotus ostreatus*, *Pleurotus pulmonarius*, *Pleurotus rickii* and *Pleurotus djamor*, the latter with three varieties: var. *djamor*, var. *cyathiformis* and var. *roseus*[13,14]. The search for new strains should also be an important point to be taken into account by researchers because it would be possible to improve yields if new more productive strains were found.

P. ostreatus has been commercially cultivated in Argentina for about 28 years. The majority of farmers (95%) use wheat straw as substrate, which is pasteurized by steam or hot water (80 °C). The highest biological efficiencies obtained vary between 100-120%; annual production is, at present, estimated in 100 ton per year, most of it (95%) is sold fresh[1]. In the last 10 years, other species of *Pleurotus* such us *Pleurotus sajor-caju* and *P. djamor* were incorporated to industrial production but, in all cases, using commercial strains from Europe and Asia. The knowledge about native strains from Argentina is scant. The study of new genotypes could have suitable advantages for mushroom production, such as the increasing of yields, variation in fruiting bodies size or the decreasing of production time. In this paper we used species of genus *Pleurotus* to evaluate the importance of finding new naturally occurring species and strains for improving biological efficiency (BE) and some morphological parameters which are important in edible mushroom production such as pileus and stem size. Five species and 14 naturally occurring strains were cultivated, BE and morphological parameters were recorded and analyzed. In addition, we evaluated the value of *P. albidus* as a new species for mushroom production.

Materials and methods

Strains used

P. albidus: Argentina, Buenos Aires, La Lucila, in canker of *Salix humboldtiana*, 5-V-1996, BAFC 2787; La Plata, on stump, III-1996, *coll. J. Deschamps*, BAFC 809; Pergamino, on *Salix* sp., 6-IV-1996, *coll. E. Albertó*, BAFC 695; Tigre, on *Populus* sp., 10-III-1997, *coll.* Claudio Lázzari, BAFC 136; Córdoba, La Punilla, on *Populus* sp., 25-II-2001, *coll. N. Manero and B. J. Lechner*, BAFC 190. *P. cystidiosus:* Argentina, Capital Federal, Barrio La Paternal, on dead zone of *Platanus* sp., *coll. S. Frachia*, 18-IV-2000, BAFC 188; Buenos Aires, La Plata, Plaza San Martín, XII-27-1994, *leg. H. Spinedi*, BAFC 73. *P. djamor* var. *djamor:* Argentina, Misiones, El Palmital del cruce, 28-V-2001, *coll. E. Albertó*, BAFC 821. *P. djamor* var. *roseus:* Argentina, Misiones, El Palmital del cruce, 28-V-2001, *coll. E. Albertó*, BAFC 815. *P. ostreatus:* Argentina, Capital Federal, 18-IV-1994, *coll. Pablo Pica*, BAFC 2034; Neuquen, Moquehue, on trunk of *Araucaria araucana*, III-1993, *coll. J. del Vas*, BAFC 120; Italy, commercial strain, IX-1993, BAFC 2067. *P. pulmonarius:* Buenos Aires, Ezeiza, on living declining tree of *Populus* sp., 25-VII-1987, *coll. J. Deschamps*, BAFC 1003; Misiones, San Pedro, on branches of *Araucaria angustifolia*, 27-V-2001, *coll. E. Albertó and O. Popoff*, BAFC 76; San Pedro, S 26° 32′, W 54° 04′, on branches of *A. angustifolia*, 27-V-2001, *coll. D. Krueger*, BAFC 213; same location, same host, same date, *coll. E. Albertó*, BAFC 263.

Optimal temperature for mycelium growth

Cultures were inoculated with 7 mm diameter cylinder in 90 mm Petri dishes containing Nobles' medium[16] and incubated in the dark at 5, 12, 20, 25, 30 and 35 °C. Growth of mycelium was measured as radio of the colony in duplicate with a ruler (0.5 mm scale).

Spawn production

It was prepared in 750 ml glass bottles filled with boiled wheat grains (*Triticum durum*) and 1% w/w calcium carbonate ($CaCO_3$). Bottles were sterilized for 1.5 hour at 121 °C, cooled and then inoculated with 1 cm diam plug of mycelium grown on Nobles' medium. Bottles were incubated at 25 °C, in the dark, with periodical shaking. Time required for spawn production was recorded.

Substrate preparation

To obtain fruiting bodies, standard methods for fruiting species of *Pleurotus* were used[22,28]. Three substrates were used based on sawdust of *Salix* sp. and wheat straw, supplemented with wheat meal and oatmeal (Table 1). Three hundred grams dry weight of each mixture was introduced into polypropylene bags of 20 × 40 cm for sawdust and 30 × 45 cm for wheat straw. Final humidity in the substrate was adjusted (w/w) to 74% accounting for the initial humidity content of substrate. Bags were stopped with cotton plugs held by PVC (polyvinyl chloride) cylinders and autoclaved at 123 °C, 1.2 Kg/cm^2, for 2 h. After cooling, the bags were inoculated with 3% (wet weight) of spawn and incubated at 25 °C in the dark until the complete colonization of the substrate.

Table 1
Substrates utilized for the obtention of fruiting bodies.

SS	
Salix sp. sawdust	77%
Wheat meal	15%
Oatmeal	5%
$CaCO_3$	3%
SW	
Wheat straw	77%
Wheat meal	15%
Oatmeal	5%
$CaCO_3$	3%
W	
Wheat straw	97%
$CaCO_3$	3%

Fruiting conditions

Six small cuts (20 mm long) were regularly made on the bag surface. Bags were then moved to a culture room (2.5 × 4.5 m) for fruiting bodies production; they were kept at 18-20 °C with 9 h light/15 h dark photoperiod (20 W fluorescent light), 75 to 85% humidity levels, and watering by spray (fog type) for 5 min every 3 h which was automatically provided.

Cropping period, crop yield and morphological traits assessment

Three to four flushes were collected during the cropping period (time lapsed between the induction day and the last harvest day) defined in 120 days. Mature fruiting bodies were collected and the following production and morphological traits were registered: A) Production: i) Primordia initiation (in days) from the start of incubation; ii) BE: fresh fruiting bodies weight (total yield)/dry substrate weight (expressed as a percentage). B) Morphological traits: i) pileus breath and length; ii) stem length. After four months under fruiting conditions, bags were dried and weighed; dry matter content loss was calculated as a percentage.

Experimental design and statistical treatments

Six bags were used for treatment. Tukey HSD test was used to determine significant differences between groups in an ANOVA. The normality and homogeneity assumptions were checked by means of KS and Bartlett tests respectively for the validity of ANOVA method.

Results

Optimal temperature for mycelium growth

The effect of temperature on mycelium growth is shown in Fig. 1. Differences in the rate of growth can be observed either

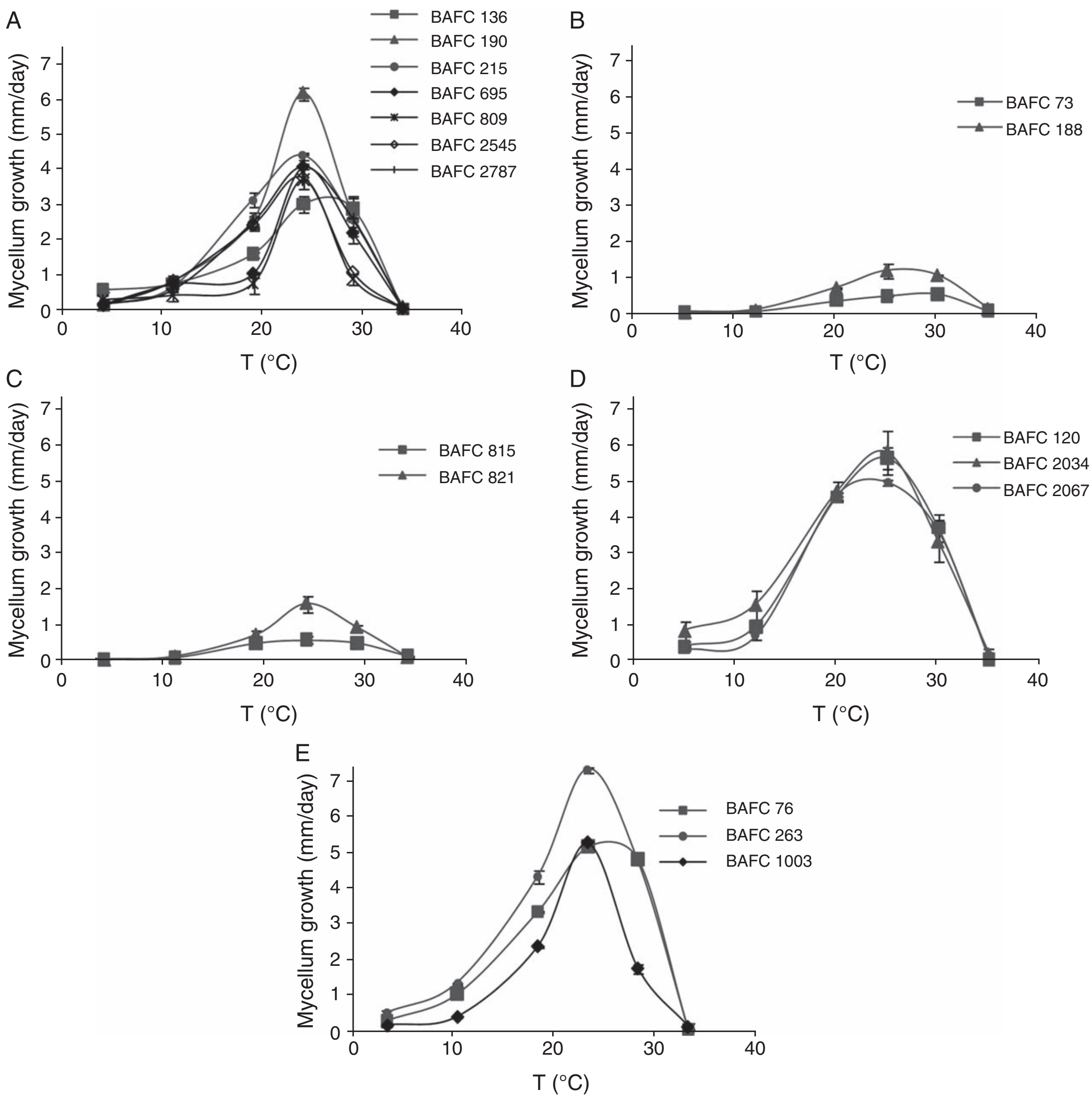

Figure 1. Effect of temperature on mycelium growth of naturally occurring *Pleurotus* species from Argentina. A) *P. albidus*. B) *P. cystidiosus*. C) *P. djamor*. D) *P. ostreatus*. E) *P. pulmonarius*.

Table 2
Time of colonization, primordia initiation, means of yield, biological efficiency (BE) and dry matter loss from *Pleurotus* strains cultivated on three different substrates.

Species	BAFC	Substrate	Time of colonization	Primordia initiation (days)	Yield	BE	Substrate dry matter loss (%)
P. albidus	136	SS	15-19	20-25	253.7	84.3 ± 15.7 ^{a}	70.3 ± 1.4 ^{ca}
		SW	15-19	29-31	397.0	132.3 ± 18.5 ^{b}	77.1 ± 5.7 ^{a}
		W	15-19	35-39	346.7	115.4 ± 14.3 ^{b}	77.4 ± 2.0 ^{da}
	190	SS	16-25	30-34	373.0	124.3 ± 15.5 ^{b}	76.1 ± 6.8 ^{a}
		SW	18	29-33	450.7	150.5 ± 15 ^{db}	79.3 ± 3.3 ^{da}
		W	18-25	34-36	485.0	161.7 ± 31.1 ^{db}	78.3 ± 3.4 ^{da}
	695	SS	25	32	268.7	89.5 ± 18.7 ^{ae}	53.3 ± 4.7 ^{e}
		SW	25	29	220.0	73.3 ± 19.5 ^{ad}	60.0 ± 1.5 ^{f}
		W	25	29-31	161.0	53.7 ± 12.2 ^{d}	64.8 ± 2.5 ^{b}
	809	SS	20	32-35	306.3	102.1 ± 16.5 ^{a}	62.0 ± 2.8 ^{bf}
		SW	19	35	218.7	74.2 ± 18.5 ^{ad}	58.5 ± 8.6 ^{b}
		W	28	40-42	269.7	89.9 ± 14.2 ^{a}	80.8 ± 4.6 ^{da}
	2787	SS	20	26-33	407.0	135.6 ± 16.5 ^{b}	85.3 ± 6.8 ^{da}
		SW	19	22-26	513.0	171.3 ± 25.0 ^{fb}	73.2 ± 5.7 ^{a}
		W	20	24-27	239.2	79.7 ± 14.0 ^{ad}	69.0 ± 1.5 ^{ca}
P. djamor	815	SS	18	35	184.7	61.6 ± 6.1 ^{a}	78.3 ± 3.7 ^{a}
		SW	18	35-37	245.3	81.8 ± 3.3 ^{a}	79.1 ± 4.4 ^{a}
		W	18	35-37	337.0	112.3 ± 19.0 ^{b}	75.0 ± 2.0 ^{a}
	821	SS	18	27	320.0	106.7 ± 16.4 ^{b}	79.7 ± 4.8 ^{a}
		SW	18	27-29	265.5	88.6 ± 16.7 ^{ab}	74.8 ± 4.5 ^{a}
		W	18	29-31	307.7	102.5 ± 15.9 ^{b}	72.7 ± 1.2 ^{a}
P. ostreatus	120	SS	27	37-40	111.0	37.0 ± 14.1 ^{a}	85.1 ± 1.1 ^{a}
		SW	27	37-40	127.5	42.5 ± 8.9 ^{a}	69.2 ± 2.6 ^{c}
		W	27	37-39	185.0	61.7 ± 6.6 ^{b}	76.4 ± 4.7 ^{b}
	2034	SS	21	35-37	203.7	67.9 ± 8.9 ^{b}	85.6 ± 4.3 ^{a}
		SW	21	32-34	371.7	122.9 ± 17.6 ^{c}	79.3 ± 1.4 ^{b}
		W	21	32-35	324.3	108.1 ± 8.4 ^{c}	80.3 ± 0.4 ^{b}
	2067	SS	21	30-32	151.7	50.5 ± 10.2 ^{ab}	82.6 ± 1.8 ^{a}
		SW	21	27-30	282.0	94.0 ± 11.9 ^{dc}	86.8 ± 3.3 ^{a}
		W	21	28-30	261.7	87.2 ± 9.1 ^{d}	84.7 ± 3.7 ^{a}
P. pulmonarius	76	SS	21	25-30	382.7	127.5 ± 18.1 ^{a}	70.7 ± 2.7 ^{d}
		SW	21	23-27	293.7	97.9 ± 18.6 ^{ae}	78.6 ± 2.6 ^{ab}
		W	21	23-30	352.3	117.4 ± 14.1 ^{ae}	76.8 ± 2.5 ^{a}
	263	SS	28	35-39	178.75	59.6 ± 7.8 ^{b}	80.0 ± 0.4 ^{b}
		SW	28	37-40	43.3	14.4 ± 0.5 ^{c}	77.0 ± 3.1 ^{ab}
		W	28	37-42	200.3	66.8 ± 13.5 ^{b}	81.4 ± 1.6 ^{b}
	1003	SS	27	35-37	272.3	94.1 ± 11.2 ^{e}	85.2 ± 1.9 ^{c}
		SW	27	35-40	384.0	128.0 ± 18.9 ^{da}	68.1 ± 0.8 ^{d}
		W	27	35-40	360.3	120.1 ± 8.0 ^{da}	70.0 ± 1.1 ^{d}

Means followed by the same letter in the same column and for the same species are not significantly different according to Tukey's test.

interespecifically or intraspecifically. The highest growth rate recorded was reached by strains BAFC 263 of *P. pulmonarius* with 7.4 mm/day which had significant differences among strains of this species ($p < 0.01$), BAFC 190 of *P. albidus*, with 6.16 mm/day which had significant differences among strains of this species ($p < 0.01$), and BAFC 2034 of *P. ostreatus* with 5.8 mm/day which had only significant differences with strain BAFC 2067. The lowest growth rate was shown by *P. cystidiosus*, followed by *P. djamor*. For the former, BAFC 188 and 73 reached hardly 1.2 and 0.5 mm/day respectively, while BAFC 821 and 815 of *P. djamor* reached 1.5 and 0.5 mm/day. Among strains of *P. ostreatus*, *P. albidus* and *P. pulmonarius* no meaningful differences were observed. *P. albidus*, *P. djamor*, *P. ostreatus* and *P. pulmonarius* reached their optimum growth at 25 °C, except strains BAFC 136 of *P. albidus* and BAFC 76 of *P. pulmonarius* which reached it at 30 °C. The optimum temperature of growth for *P. cystidiosus* was 30 °C. Strains BAFC 136, 215, 695, 809, 2545, 2787 of *P. albidus* grew 29 to 50% less than strain BAFC 190. No meaningful differences were obtained between BAFC 2034 and 120 of *P. ostreatus*, while BAFC 2067 grew 14% less than BAFC 2034. Strain BAFC 263 of *P. pulmonarius* grew 28% more than BAFC 76 and 1003.

Strains of *P. albidus*, *P. ostreatus* and *P. pulmonarius*, which had highest growth rate on Nobles' medium, required shorter time for spawn production: 8 to 12, 8 to 10 and 5 to 10 days respectively. Strains of *P. djamor* and *P. cystidiosus* were slower and required 11 to 12 days and 15 to 20 days respectively to completely colonize grains.

The range of time required for incubation (time of colonization) on different substrates at 25 °C also depended on species and varied from 15 to 45 days. Strains of *P. albidus*, *P. ostreatus, P. djamor* and *P. pulmonarius* required 15 to 20 days, meanwhile strains of *P. cystidiosus* required from 28 to 45 days (data not shown).

Primordia initiation

P. albidus required 20 to 42 days after spawning for primordia development, *P. djamor*, 27 to 37 days, *P. ostreatus* 28 to 42 days, and *P. pulmonarius* 25 to 40 days (Table 2). *P. cystidiosus* needed more than 45 days (data not shown).

Optimal substrates, production and quality traits

The effect of different substrates on fruiting bodies production of naturally occurring strains was determined (Table 2). The most suitable combinations of substrate and strain were obtained with *P. albidus* BAFC 2787 on SW, and BAFC 190 on W, which reached a BE of 171.3 and 161.7% respectively, being the highest yields obtained in this study. Regarding the other strains of *P. albidus*, the statistical analysis showed that there were significant differences ($p \leq 0.05$) among strains BAFC 190, 809 and 695 but no significant differences ($p > 0.05$) were found among substrates. When the morphology of fruiting bodies obtained with *P. albidus* was studied (Table 3), we observed that no significant differences were found between width

Table 3
Morphological properties of basidiocarps obtained from *Pleurotus* strains.

Species	BAFC	Substrate	Width of pileus	Length of pileus	Length of stem
P. albidus	136	SS	6.4 ± 1.7 a	5.3 ± 1.8 a	1.8 ± 0.6 ba
		SW	5.9 ± 1.9 a	4.9 ± 1.4 a	2.5 ± 0.8 a
		W	5.4 ± 1.3 a	4.3 ± 1.3 a	2.0 ± 0.9 a
	190	SS	5.9 ± 1.7 a	4.2 ± 1.1 a	2.3 ± 1.0 a
		SW	6.2 ± 1.4 a	5.5 ± 1.4 a	2.8 ± 1.1 a
		W	5.5 ± 1.7 a	4.4 ± 1.4 a	3.0 ± 1.0 a
	695	SS	5.4 ± 1.5 a	4.8 ± 1.4 a	2.0 ± 1.0 a
		SW	7.3 ± 2.7 a	6.0 ± 1.7 a	2.4 ± 1.0 a
		W	5.6 ± 2.2 a	4.8 ± 1.4 a	2.1 ± 1.0 a
	809	SS	5.7 ± 1.7 a	4.9 ± 1.7 a	2.3 ± 1.3 a
		SW	7.1 ± 3.3 a	6.1 ± 2.4 a	2.9 ± 1.5 a
		W	5.5 ± 3.5 a	5.2 ± 3.6 a	3.4 ± 1.4 a
	2787	SS	6.1 ± 1.6 a	5.4 ± 2.0 a	2.3 ± 1.0 a
		SW	6.0 ± 1.7 a	5.3 ± 2.0 a	3.9 ± 1.4 ca
		W	7.7 ± 2.6 a	5.6 ± 2.4 a	3.2 ± 1.1 a
P. djamor	815	SS	6.9 ± 1.2 ca	6.3 ± 2.6 a	1.9 ± 0.3 a
		SW	8.0 ± 2.7 a	6.8 ± 1.6 a	1.6 ± 0.5 a
		W	9.6 ± 1.4 ba	7.1 ± 2.2 a	0.7 ± 0.5 a
	821	SS	5.7 ± 2.1 ca	4.1 ± 1.3 ba	1.8 ± 0.2 a
		SW	6.9 ± 1.6 a	8.0 ± 2.4 ca	2.0 ± 0.4 a
		W	6.7 ± 2.5 a	6.4 ± 3.3 a	0.8 ± 0.3 a
P. ostreatus	120	SS	8.1 ± 3.0 a	7.7 ± 2.3 a	3.3 ± 1.0 a
		SW	10.5 ± 1.5 ba	9.7 ± 2.1 ba	4.8 ± 0.8 ba
		W	6.7 ± 1.8 a	6.7 ± 2.4 a	3.6 ± 1.3 a
	2034	SS	8.0 ± 3.3 a	6.7 ± 2.3 a	2.3 ± 1.4 ca
		SW	6.5 ± 2.4 ca	5.6 ± 1.7 ca	3.6 ± 1.4 a
		W	6.7 ± 2.6 a	6.7 ± 2.3 a	3.6 ± 1.5 a
	2067	SS	5.4 ± 1.6 ca	5.1 ± 1.0 ca	2.2 ± 0.7 ca
		SW	6.2 ± 1.6 a	6.2 ± 1.4 a	2.3 ± 0.6 ca
		W	7.0 ± 1.6 a	6.1 ± 0.7 a	3.2 ± 0.7 ca
P. pulmonarius	76	SS	6.3 ± 1.9 a	4.4 ± 1.2 a	1.0 ± 0.3 ba
		SW	6.7 ± 2.1 a	5.2 ± 1.6 a	1.1 ± 0.8 a
		W	6.9 ± 2.3 a	5.2 ± 1.6 a	1.5 ± 0.7 a
	263	SS	5.9 ± 1.5 a	4.6 ± 1.2 a	1.5 ± 0.8 a
		SW	6.8 ± 2.3 a	4.9 ± 1.6 a	1.9 ± 0.9 a
		W	6.4 ± 2.0 a	5.0 ± 1.5 a	1.7 ± 2.3 a
	1003	SS	7.3 ± 2.7 a	6.1 ± 2.3 a	1.7 ± 0.6 a
		SW	7.4 ± 2.3 a	5.9 ± 1.8 a	1.9 ± 0.8 a
		W	7.9 ± 2.5 a	6.2 ± 1.7 a	2.1 ± 0.6 ca

Means followed by the same letter in the same column and for the same species are not significantly different according to Tukey's test.

and length of pileus. Strain BAFC 2787 produced the largest stems on SW. Only significant differences ($p \leq 0.05$) for stems length were found between BAFC 2787 on SW and BAFC 136 on SS.

BEs values of *P. djamor* strains varied from 61 to 112% (Table 2). Strains BAFC 815 on W, and 821 on SS and W, reached the highest BE with significant differences compared with the other treatments for the same strains. The widest pileus was obtained with strain BAFC 815 on W showing only significant differences with SS for both strains. When the length of pileus was compared, we found that it was minor for BAFC 821 on SS but only significant differences were obtained between SS and SW. No significant differences were observed when stem length was compared (Table 3).

The best yield of *P. ostreatus* was produced by BAFC 2034 on SW reaching BE 122.9 (Table 2). Similar values were obtained with BAFC 2434 on W and 2067 on SW. The analysis of BEs of *P. ostreatus* strains showed that BAFC 120, which developed several aborted primordia, produced lowest yields (BE 37 to 61.7). For this species, SS produced the lowest BE, showing significant differences with W and SW. The study of morphology showed that largest sizes of pileus were obtained with the strain BAFC 120 on SW. The highest stem size was produced by strain BAFC 120 on SW and showed significant differences ($p \leq 0.05$) with strain BAFC 2034 on SS and BAFC 2067 on all substrates (Table 3).

Yields obtained with strains of *P. pulmonarius* were very variable. The best yield of this species was produced by strains BAFC 76 and 1003 (BE 97.9 to 128%) showing significant differences ($p \leq 0.05$) with BAFC 263 which produced lowest yield in this species (Table 2). The morphological study showed that no significant differences in the size of the pileus were found among strains when cultivated in different substrates. Stems obtained with strain BAFC 76 on SS were significantly shorter than those obtained with BAFC 1003 on W.

Yields of *P. cystidiosus* were very poor with BE values of 0-30%; these were the lowest reached in this study (data not shown). SW did not produce fruiting bodies. Due to the low yields obtained with this species, morphology traits where not studied.

Dry matter loss

Dry matter loss for *P. albidus* varied from 53.3 ± 4.7 on SS for strain BAFC 695 to 85.3 ± 6.8 on SS for BAFC 2787 with significant differences ($p < 0.05$). *P. djamor* did not show significant differences in dry matter loss varying from 72.7 ± 1.2 on W to 79.7 ± 9.8 on SS for BAFC 821. *P. ostreatus* varied from 69.2 ± 2.6 for BAFC 120 to 86.8 ± 3.3 for BAFC 2007 both growing on SW with significant differences ($p < 0.05$). For *P. pulmonarius* the loss varied from 68.1 ± 0.8 on SW to 85.2 ± 1.9 on SS both for strain 1003 with significant differences ($p < 0.05$). Dry matter loss of the substrates was generally in agreement with BE increase (Table 2). Nevertheless, several strains with low BE, such as BAFC 120 (on all substrates), 2067 (on SS) of *P. ostreatus* and BAFC 263 (on SW) of *P. pulmonarius*, showed high dry matter loss (Table 2). Also strains with high yields had comparatively lower substrate dry matter lost as BAFC 809 on SS or 2787 on SW.

Discussion

In this work we studied the capacity of naturally occurring strains of *Pleurotus* to produce fruiting bodies in three different substrates and their morphology variation. Strains assayed belonged to different geographic areas and were adapted to different climate conditions and substrates[13]. Thus, we studied the optimal temperature of growth in order to optimize and reduce spawning run time. When growth rate was compared for each strain among the temperatures assayed, we observed that the strains of *P. albidus*, *P. ostreatus* and *P. pulmonarius,* which belong to "*P. ostreatus* clade"[2], had the highest rate growth and grew 51.9 to 93.2% more than strains of *P. djamor* and *P. cystidiosus* respectively. Zadrazil[28] studied commercial strains of *P. ostreatus* and *P. florida (= P. pulmonarius)* and found a rate growth higher than naturally occurring strains herein assayed. Anyway, no relationship was found when optimal mycelium growth, incubation time and yields were compared. We expected that strains with high speed of growth would have shorter spawning run time but this did not happen. While *P. albidus* BAFC 190 had the highest speed of growth on agar medium and BAFC 136 had the lowest, both species needed similar time to complete substrate colonization (Table 2). Growth rate of *P. djamor* strains (fig. 1) was very low in agar (up to 2 mm/day) compared with results reported by Salmones et al[21]. The low growth in Nobles' medium (fig. 1) contrasted with a faster growth obtained during the incubation period on the three different substrates assayed, since they only needed 18 days for a complete colonization of substrates (Table 2). Strains of *P. ostreatus* BAFC 120 and *P. pulmonarius* BAFC 263 had the highest speed of growth on agar but needed 21 and 27 days to complete substrate colonization. Thus, time required for naturally occurring strains to colonize substrates can not be predicted based on assays made on agar Nobles' medium.

Primordia initiation in *Pleurotus* species required 21 to 30 days after inoculation[19,20] while in the present study it required 20 to 42 days. We observed that 4 of the 5 strains of *P. albidus* assayed on supplemented straw required shorter time for primordia formation than the same substrate without supplements. This effect was not observed in the other species. Uhart et al[24] found that supplemented substrates reduced the time of first harvest day in *Agrocybe cylindracea*. Apparently, some nutrients could help to reduce time required for the primordia formation.

The BE strongly depended on the strains used. For each species tested, it was possible to select a high productive strain, with the exception of *P. cystidiosus*. The poor BE obtained with this species could be attributed to strains type, resulting in strains of low production or to substrates formulation; according to Stamets[22], strains of *P. cystidiosus* from Thailand and Taiwan produced high crops on rice straw and low yields on wheat straw, being the strains more narrowly specific in their fruiting requirements.

It was possible to find a strain of *P. ostreatus* with better BE that the commercial strain BAFC 2067. Indeed, strain BAFC 2034 produced 30% more than strain 2067 with fruiting bodies similar in shape and size. It was also possible to exceed in 82% the yield obtained in SW for the commercial strain with the naturally occurring strain 2787 of *P. albidus* on the same substrate.

Mushroom farmers are not only interested in obtaining high yields but also in offering a good product of high quality where the morphology of fruiting bodies is also important. Colour, size of pileus and stems are also imperative facts taken into account when farmers select the spawn. In this work, different strains and substrates were tested to evaluate the changes produced in three morphological parameters. We have observed that in the case of *P. albidus* (five strains) and *P. pulmonarius* (three strains) the size of the pileus did not vary with different treatments. In contrast some minor but significant differences were found among strains of *P. djamor* and *P. ostreatus*. In the case of BAFC 120 although larger specimens were produced, yields have been low.

The length of stem is an important factor for mushroom farmers because when stems are too long they need to be cut and discarded. As a consequence, shorter stems are preferred. In general, low variations in the length of the stem were found. The strain 1003 of *P. pulmonarius* on SW was very interesting because it produced high BE with short stems. On the other hand, the most productive strain, BAFC 2787 on SW, produced high crops but with longer stems. Apparently, the morphology of strains is not drastically altered when cultivated in different substrates.

In general, wild strains had a good performance on W or SW which showed the highest BE. This is in agreement with previous studies in which straw was reported to be a good substrate for cultivation of *Pleurotus* species[3,7,25]. This is an important fact because wheat straw is abundant in the region and is preferred by mushrooms growers because it allows a process of pasteurization at 60 °C while the use of sawdust requires a more aggressive and expensive treatment such as the sterilization by steam. The use of straw could be a favorable factor for growers to adopt these strains in their farms.

Generally, the dry matter loss is in agreement with mushroom yield and biological efficiency. It was recently proved for *Polyporus tenuiculus*[17] a new mushroom that can be industrially cultivated. According to Zhang et al[30] the dry matter loss of the substrate (rice and wheat straw) for *P. sajor-caju* varied from 30 to 44% measured after 40 days. In our experiment dried matter loss reached 53 to 87% after 120 days of cultivation. The increase of dry matter loss could be due to a longer cropping period or due to a major biodegradation activity of the strains. Dry matter loss is partially assimilated into the mushroom fruiting bodies or mycelium and partly lost into the atmosphere as carbon dioxide due to mushroom respiration.

In this experiment we used a 120-day period of cropping as we did not know how naturally occurring strains could behave in a mushroom farm and we did not want to lose yield. We observed that this period was too long and that a shorter period of 90 days was enough for the evaluation of these strains.

Sawdust of *Salix* and wheat straw resulted good substrates for fruiting *Pleurotus* species. We found that strain 2787 on SW of *P. albidus* produced higher BE than the commercial strain of *P. ostreatus* used. Even more, the BE obtained for this strain is one of the highest ever reached for a *Pleurotus* species assayed in the most diverse substrates[15]. One of the most important objectives of research in mushrooms science is to achieve higher yields transforming agriculture waste into high quality food. Based on our results, to improve yields, it is interesting not only to evaluate substrates but also to use naturally occurring strains. The isolation of new strains and their preservation in culture collections will also be useful for the safeguarding of germplasm. Because of the high yields and the good quality of mushrooms obtained, we propose *P. albidus* as a new species for intensive industrial cultivation. This species, characterized by the white, circular to infundibuliform pileus with a margin entire to lacerate-crenate[2] requires culture conditions similar to those of *P. ostreatus*. It will allow farmers to cultivate it without new requirements or larger investments allowing for the expansion of the variety of product offering.

Conflict of interest

Authors have no conflict of interests.

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