Selection of strains of *Lentinula edodes* and *Lentinula boryana* adapted for efficient mycelial growth on wheat straw

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Summary

Mycelial growth rates are presented for 11 strains of *Lentinula edodes* and six strains of *Lentinula boryana* cultivated on solid media: derived from malt extract (MEA); malt yeast extract (YMEA); and, YMEA plus soluble lignin derivatives (YMEA+WSLD). The results were compared with data for mycelial growth rates, of the same strains cultivated on substrates derived from wheat straw treated at different temperatures (50, 65, 75 and autoclaving at 121 °C). In general, the addition of WSLD significantly reduced mycelial growth rates in both species. The greatest mycelial growth rate was obtained on sterilized straw at 121 °C for the majority of strains. However, this growth was not significantly different from that obtained at 75 °C. *L. edodes* showed greater growth rates than *L. boryana*. The feasibility of using estimates of mycelial growth rate on YMEA and YMEA+WSLD are discussed as possible indicators of a strain's potential for mycelial growth on substrates derived from wheat straw.

Key words

Lentinula edodes, *Lentinula boryana*, Shiitake, Strain selection, Mycelial growth, Wheat straw

Selección de cepas de *Lentinula edodes* y *Lentinula boryana* adaptadas para un eficiente crecimiento micelial sobre paja de trigo

Resumen

Se presentan los resultados del crecimiento micelial de 11 cepas de *Lentinula edodes* y séis cepas de *Lentinula boryana* en medios de cultivo sólidos a base de extracto de malta (MEA), extracto de malta y levadura (YMEA) y YMEA adicionado de derivados solubles de lignina (YMEA+WSLD), en comparación con el crecimiento micelial de las mismas cepas en un substrato a base de paja de trigo tratada a diferentes temperaturas (50, 65, 75 y esterilización a 121 °C). En general la adición de WSLD disminuye significativamente el crecimiento micelial en ambas especies. Para la mayoría de las cepas, el mayor crecimiento micelial se obtuvo en la paja tratada a 121 °C, sin embargo, dicho crecimiento no fue significativamente diferente del obtenido a 75 °C. Las cepas de *L. edodes* muestran un mayor crecimiento que las de *L. boryana*. Se discute sobre factibilidad de utilizar las estimaciones de crecimiento micelial en YMEA y YMEA+WSLD como indicadores de las potencialidades de crecimiento micelial de una cepa en un substrato a base de paja de trigo.

Palabras clave

Lentinula edodes, *Lentinula boryana*, Shiitake, Selección de cepas, Crecimiento micelial, Paja de trigo

The capacity of a mushroom to grow on a lignocellulosic substrate is related to the vigor of its mycelium, as well as to its capacity to activate physiological mechanisms necessary to adequately exploit the medium [1]. If fructification characters are among the criteria for determining strain selection, particular interest must be placed on the strain capacity to invade a given substrate. The first important stage in the cultivation of a mushroom on a solid substrate is the speed of hyphal colonization. Once the mycelium has spatially occupied the substrate, it will also need to utilize the nutrients it encounters. Initial colonization speed is even more important when a non-sterile substrate is utilized because of the presence of antagonistic microorganisms.

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In the case of wheat straw, the possibility of colonization and nutritive resource utilization is related to the production and liberation of hydrolytic and oxidative enzymes by the mushroom. Such enzymes are capable of transforming the complex lignocellulose matrix into compounds of low molecular weight that will be easily assimilated [2]. This principle of nutritive adaptation to the substrate plays an important role in the commercial cultivation of edible mushrooms species, where the aim is to provide the mushroom with a decisive advantage in colonizing the substrate before its competitors [3]. The traditional method of cultivating the shiitake in logs has been partially displaced by the use of substrates based upon enriched sterilized sawdust [4–6]. With the aim of reducing production time and substrate sterilization costs for the cultivation of the shiitake, Delpech and Olivier [7] proposed the use of a pasteurized substrate (heat treatment at 65 °C) formed from wheat straw. This method has been adopted by European cultivators and little by little it is gaining adepts in the USA [8]. Due to chemical and structural differences in the cultivation substrates, as well as thermal treatment, the selection of genotypes adapted to these conditions is critically important to ensure a good production of fruiting bodies in the shortest time possible.

The objective of this work is to comparatively study the mycelial growth ability of strains of two species of *Lentinula* in wheat straw treated at different temperatures, as well as in solid cultivation media, in order to determine the adaptive capacity of the strains to this substrate and to propose a means for estimating this capacity with standardized methods.

MATERIALS AND METHODS

Strains and culture medium. Seventeen strains ascribed to the genus *Lentinula* were studied. Eleven of these strains, belonging to *Lentinula edodes* (shiitake), are deposited in the Strain Collection of the Institut National de la Recherche Agronomique, INRA, Bordeaux, France (INRA Bxc) and include French commercial strains, strains from international collections, and hybrids obtained in laboratory crosses. The six remaining strains ascribed to the American species *Lentinula boryana*, are deposited in the Strain Collection at the Instituto de Ecología, Xalapa, Mexico (IE). Mycelial growth was studied by using three solid culture media, as well as a substrate derived from wheat straw, but treated under different temperature regimes. Culture media employed were malt extract (MEA: 1% malt extract, 1.5% agar), yeast malt extracts (YMEA: 2% malt extract, 0.2% yeast extract, 1.5% agar), and yeast malt extracts with added soluble lignin derivatives (YMEA+WSLD) obtained from an infusion of Indulin AT (Sigma: Alkali lignin, USA). This last culture medium was prepared according to Mata *et al.* [9], phenol concentration was measured according to the method of Box [10] and adjusted to 1 mM. After autoclave sterilization (121 °C / 15 min), 20 ml of the culture medium was placed in Petri dishes (90 mm diameter). Samples were inoculated with mycelial disks (5 mm diameter) pre-cultivated on MEA. Two successive cultures were prepared with the YMEA and YMEA+WSLD media. From the first one, called Culture 1 (C1), a mycelial disk was taken off to inoculate the second culture (C2). Ten samples were prepared for each culture medium and each strain. Samples were incubated for seven days in darkness at 25°C. Mycelial growth was estimated by measuring the diameter of the mycelia along two perpendicular axes. Changes observed in mycelial growth rates between C1 and C2, when grown upon YMEA and YMEA+WSLD media, were considered estimates of the adaptability of each strain to phenolic compounds [9].

Mycelial growth on wheat straw. Mycelial growth ability was studied using a wheat straw substrate subjected to different heat treatments (50, 60, 75 and 121 °C). Substrate was prepared according to Delpech and Olivier [7]. Plastic bags containing 60 g of moistened straw with 10% of gypsum were placed in a oven and heat treated at 50, 65 and 75°C for a 24 h period. The bags for the 121°C treatment were autoclaved for 1 h. After cooling, substrate was placed in sterile Petri dishes, with the cover perforated at five places (in order to allow a good air exchange), at a rate of 7 g of substrate / dish. Substrate was inoculated with four mycelial discs, previously cultivated on MEA, distributed evenly over the surface of the substrate. Three replicates of each treatment were prepared per strain, sealed with alimentary film and incubated for four days at 25 °C in total darkness. Surface area of mycelial growth (cm^2) was measured using a digitizing pad.

Studies were also carried out, using a second batch of straw substrate, with six strains of *L. edodes* that had been selected according to the results of the previous investigation. These strains were M115, V084 and 4055, plus three hybrid strains obtained from the cross V084 x 4055. Hybrid strains were designated A9, A10 and B19. Straw used in this part of the experiment had been stored covered to protect it from the rain, for three years. Based upon previous results, it was decided to only study the treatment at 65 °C and the sterilization treatment.

Statistical analysis of the data. Data on colony diameters and areas, both on the MEA medium and on the wheat straw substrate, were treated with an analysis of variance using the Tukey multiple comparisons method (P = 95%) to determine the similarity between media. With respect to mycelial growth on the YMEA and YMEA+WSLD media in the C1 and C2 cultures, pairwise comparisons were undertaken to establish treatment effects using the Student's *t*-test at the 5% level of rejection. Linear regression analysis by the least squares method was used to determine correlation between the treatments. Correlations were calculated between mycelial growth for each strain and the different temperature treatment on straw-based substrate, and between mycelial growth obtained on the culture media and on the straw-based substrate.

RESULTS

Growth on MEA medium. Mycelial diameter of *L. edodes* strains after seven days varied between 4.9 and 7.1 cm (Table 1). Strain Q616 grow slowly with a diameter of 4.9 cm whilst M115 and V084 grew rapidly with diameters greater than 7 cm. The remaining strains had diameters between 5.9 and 6.8 cm. *L. boryana* strains had mycelial diameters ranging from 4.4 to 5.4 cm. Although some strains of *L. boryana* showed greater growth than some strains of *L. edodes*, the average diameter of the eight strains of *L. edodes* studied in this part of the investigation was greater than the average calculated for *L. boryana* strains (Tables 1 and 2). Statistical analysis of the data showed significant differences between the strains of both species.

Growth on YMEA and YMEA+WSLD media. Independent of quantitative variations between strains, the addition of WSLD in the medium decreased the mycelial growth rates in cultures C1 and C2 for nine of the 11 strains of *L. edodes* and all strains of *L. boryana* (Tables 1 and 2). Specifically, in C1 and C2 average mycelial diameter of *L. edodes* was reduced by 17 % and 13% respec-

Table 1. Diameter of mycelia of *Lentinula edodes* grown on MEA, YMEA and YMEA+WSLD, after seven days of incubation at 25 °C.

Strains	MEA	YMEA		YMEA + WSLD	
		C1	C2	C1	C2
Q 601	6.2 ± 0.11 bcd [1]	3.8 ± 0.25	4.3 ± 0.16	3.4 ± 0.13	3.8 ± 0.26
Q 613	6.5 ± 0.04 def	3.4 ± 0.22 [4]	3.9 ± 0.15	3.4 ± 0.12 [4]	3.2 ± 0.45
Q 616	4.9 ± 0.04 a	4.5 ± 0.09	4.7 ± 0.16	[3] 3.6 ± 0.09	[3] 3.6 ± 0.07
MS 20	6.0 ± 0.03 bc	3.9 ± 0.13	4.8 ± 0.26	3.5 ± 0.18	4.0 ± 0.24
M 115	7.0 ± 0.02 gh	[2] 5.0 ± 0.39	[2] 5.0 ± 0.22	3.9 ± 0.23	4.9 ± 0.16
V084	7.1 ± 0.05 hi	[2] 4.4 ± 0.75 [4]	[2] 4.6 ± 0.34 [5]	[3] 4.3 ± 0.22 [4]	[3] 4.4 ± 0.12 [5]
4055	6.8 ± 0.03 fg	[2] 4.5 ± 0.15	[2] 4.6 ± 0.19	3.4 ± 0.20	3.9 ± 0.18
4068	5.9 ± 0.04 b	[2] 3.6 ± 0.15	[2] 3.6 ± 0.17	[3] 3.2 ± 0.11	[3] 3.1 ± 0.15
A 9		4.2 ± 0.84	5.1 ± 0.60 [5]	3.6 ± 0.99	4.8 ± 0.27 [5]
A 10		5.0 ± 0.14	5.2 ± 0.20	3.6 ± 0.22	4.6 ± 0.24
B 19		4.1 ± 0.18	5.2 ± 0.18	2.9 ± 0.24	4.5 ± 0.15
$\bar{X}$	6.3	4.2	4.6	3.5	4.0

1 = Means ± standard deviations for three replicates. When followed by the same letters, the means were not significantly different (P= 0.05)
2 = without significant differences between the C1 and C2 cultures on YMEA
3 = without significant differences between the C1 and C2 cultures on YMEA+WSLD
4 = without significant differences in the C1 culture on YMEA and YMEA+WSLD
5 = without significant differences in the C2 culture on YMEA and YMEA+WSLD

Table 2. Diameter of mycelia of *Lentinula boryana* grown on MEA, YMEA and YMEA+WSLD, after seven days of incubation at 25 °C.

Strains	MEA	YMEA		YMEA + WSLD	
		C1	C2	C1	C2
IE 17	4.4 ± 0.45 a [1]	3.5 ± 0.13	3.7 ± 0.14	2.6 ± 0.07	3.2 ± 0.14
IE 67	4.8 ± 0.16 b	2.9 ± 0.15	3.9 ± 0.17	2.6 ± 0.28	2.9 ± 0.11
IE 93	5.4 ± 0.39 c	3.3 ± 0.15	3.7 ± 0.30	3.0 ± 0.07	2.8 ± 0.27
IE 152	5.4 ± 0.14 c	[2] 3.5 ± 0.11	[2] 3.4 ± 0.13	2.6 ± 0.08	3.0 ± 0.15
IE 154	4.7 ± 0.33 b	2.6 ± 0.17	2.8 ± 0.07	1.9 ± 0.09	2.5 ± 0.07
IE 155	4.4 ± 0.36 a	3.4 ± 0.21	3.5 ± 0.22	2.7 ± 0.08	3.0 ± 0.13
$\bar{X}$	4.9	3.2	3.5	2.6	2.9

1 = Means ± standard deviations for three replicates. When followed by the same letters, the means were not significantly different (P=0.05)
2 = without significant differences between the C1 and C2 cultures on YMEA
3 = without significant differences between the C1 and C2 cultures on YMEA+WSLD
4 = without significant differences in the C1 culture on YMEA and YMEA+WSLD
5 = without significant differences in the C2 culture on YMEA and YMEA+WSLD

tively. Whilst *L. boryana* had a reduction of 26% and 17% respectively (Tables 1 and 2). However, mycelial diameter at seven days was significantly higher in C2 than in C1 on YMEA for seven strains of *L. edodes* and five strains of *L. boryana*. On the YMEA+WSLD medium, this significant increase was seen for eight strains of *L. edodes* and six strains of *L. boryana*. It is interesting to note that the mycelial diameter of *L. edodes* strain V084 was not significantly different in both culture media.

Mycelial growth on wheat straw. On straw-based substrate, the mycelial area for the majority of the strains tended to increase with increased temperature treatment of the substrate (Figures 1 and 2). However, in six of eight strains of *L. edodes* and five of six strains of *L. boryana*, differences between the treatment at 75°C and sterilization by autoclaving were not significant. In the same way, the difference between the treatments at 50 and 65°C was not significant for the majority of strains. Correlations among the four treatments were highly significant for *L. boryana*, the better correlation being between 75 and 121 °C. The only significant correlation for *L. edodes* was between the 75 and 121 °C treatments (Table 3).

The greatest growth rate was obtained with *L. edodes* strain V084, with a surface area of 4.3 cm^2 at four days, whereas this strain had a low growth rate in the 65 °C treatment. In contrast, the M115 strain had an important initial growth ability on straw treated at 65 °C with 3.3 cm^2 after four days of incubation. These two strains belong to a group known for rapid growth on MEA. Among the French commercial strains studied, in addition to the V084 strain, the 4055 and 4068 strains presented regular mycelial growth under all the treatments (Figure 1). Greatest initial growth of *L. boryana* strains was found with the IE 154 strain, however a surface area of only 2.8 cm^2 was attained on sterilized straw. The IE 17 strain only grew on straw that had been treated at 75 °C or sterilized at 121 °C and its growth was particularly slow (Figure 2).

The results using the second batch of straw substrate with six strains of *L. edodes* (Figure 3), showed an effect due to the thermal treatment of the substrate that is less clear than that seen in figure 1. Greatest mycelial growth rate on sterilized straw was observed for the M115 and A9 strains, with 5.8 and 5.5 cm^2, respectively, but none of these showed significant differences from the growth obtained on straw treated at 65 °C. The 4055 strain always showed scanty growth, with no significant differences between the two treatments. With respect to the hybrids, the greatest growth rates were obtained on the sterilized substrate and were always higher than that of the parent strains.

Correlations between mycelial growth abilities on the solid medium and on the straw-based substrate. Correlations were tested between growth diameters attained on solid medium and mycelial area achieved on straw-based substrate treated at different temperatures. Results are shown in Table 4. Data from eight strains of *L. edodes* and six strains of *L. boryana* on MEA, showed significant correlations with the data on straw that had been treated at 75 and 121°C. Significant correlations were also found for the *L. edodes* strains between mycelial diameters on YMEA+WSLD medium and colony areas on straw treated at 65, 75 and 121°C.

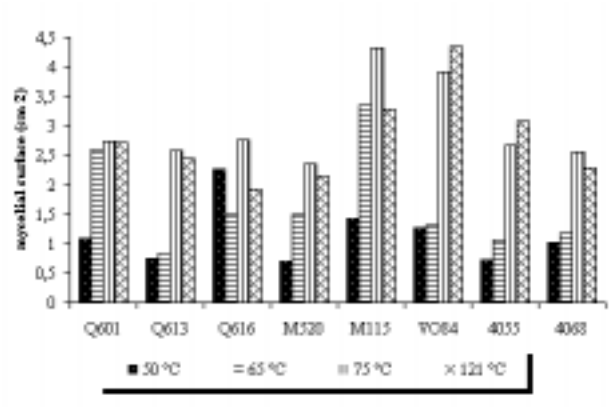

Figure 1. Mycelial surface areas for *L. edodes* strains after growth for four days on wheat straw treated at different temperatures.

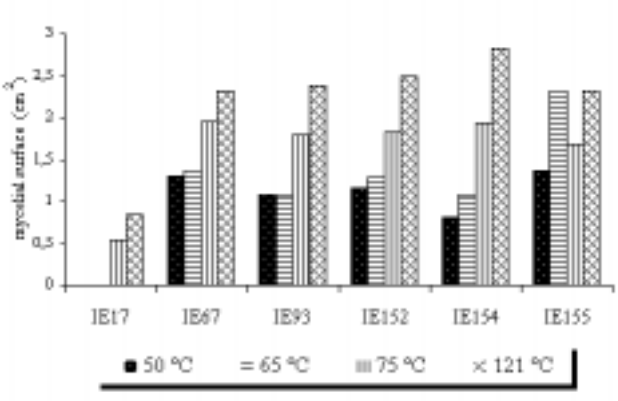

Figure 2. Mycelial surface areas for *L. boryana* strains after growth for four days on wheat straw treated at different temperatures.

Table 3. Correlation coefficients between mycelial areas on substrates derived from wheat straw treated at different temperatures.

Species	Paired temperature treatment					
	50-65	50-75	50-121	65-75	65-121	75-121
L. boryana	0.895	0.871	0.787	0.686	0.668	0.967
L. edodes	0.286	0.286	-0.09	0.594	0.178	0.762

DISCUSSION

The existence of a genetic variability and its conservation are necessary conditions in selection work. In this study, the majority of the *L. edodes* and *L. boryana* strains tested grew satisfactorily in culture media of simple composition. This is an essential condition in order to maintain strains in the collection and utilize them in the preparation of inocula for cultivation on lignocellulosic substrates. This work has demonstrated variability in the aptitude for mycelial growth between strains of *L. edodes*, and between *L. edodes* and *L. boryana* species. Strains of *L. edodes* showed, in general, a greater rate of initial growth than strains of *L. boryana*, both in the culture media studied (MEA, YMEA, YMEA+WSLD) and in the substrate derived from wheat straw. This initial growth was accompanied by a better adaptation to the straw-based substrates treated at 65 °C. The difference in behavior between these two species is probably the cause of difficulties encountered in previous attempts to cultivate *L. boryana* under conditions which are better suited for the cultivation of *L. edodes* [9, 11].

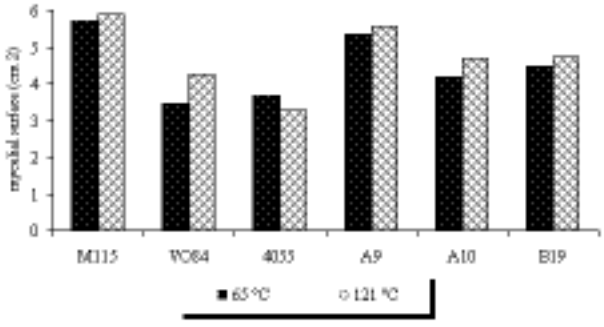

Figure 3. Mycelial surface areas for *L. edodes* strains after growth for four days on wheat straw treated at two different temperatures.

Table 4. Correlation coefficients between mycelial diameters on three solid culture media and mycelial areas on straw treated at different temperatures.

Species	Culture medium	Treatment temperature for the straw			
		50 °C	65 °C	75 °C	121 °C
L. boryana	MEA	0.375	0.01	0.491	0.454
	YMEA	0.056	-0.09	-0.28	-0.5
	YMEA+WSLD	-0.3	-0.21	-0.69	-0.78
L. edodes	MEA	-0.52	0.186	0.592	0.829
	YMEA	0.329	0.499	0.491	0.287
	YMEA+WSLD	0.161	0.677	0.821	0.654

Data for the YMEA and YMEA+WSLD media obtained from C2 culture.

Recent studies on mycelial growth in *L. edodes*, using sterilized or "pasteurized" substrates, has focused on wood-based, compound substrates and treatment temperatures near 100 °C [12–16]. Few studies have considered the use of straw [4,5,7,17] and other byproducts of agricultural activities [18]. The results of this study shows that changes in the quality of the wheat straw substrate produced between 65 and 75°C can affect the mycelial growth rate of *L. edodes* and *L. boryana*, as no differences were detected on substrates treated at 75°C or sterilized. Differences encountered in mycelial growth rates could be related to a change in the biochemical quality of the substrate, and at the same time, to biotic factors that varied significantly between substrates treated at 65 and 75°C. For example, in compost prepared from wheat straw and utilized for cultivating the white button mushroom, *Agaricus bisporus*, actinomycetes and filamentous fungi were killed when temperature in the compost was increased from 65 to 79 °C [19].

Differences in results between the two batches of straw (each one with a different storage time) might equally be related to natural sources of variation in the straw. Various factors play important roles in the final quality of wheat straw used in the cultivation of edible mushrooms. Among the most important factors are: 1) prevailing climatic conditions during the cultivation of the wheat crop, 2) genotypic variation in the wheat

variety, 3) environmental variation attributable to cultivation site and management practices carried out during crop production, 4) the type of harvest, and 5) storage conditions [20]. The chemical composition and degradability of straw can also be influenced by the quantity and quality of fungicides utilized during wheat cultivation [21], storage time of the harvest [7], and the wheat cultivar chosen as a source for straw [22].

In the context of variation among the straw samples used, mycelial growth abilities will have to be taken into account in a semi-synthetic media in order to develop a more reliable methodology for strain selection. According to Jongbloed and Borst-Pauwels [23], radial growth reflects resource exploitation, whereas biomass production is a measurement of the accumulation of carbon and nutrients. Under the experimental conditions of the present work, mycelial diameters of *L. edodes* strains on the MEA and YMEA+WSLD media for culture 2 (C2) showed positive correlations with mycelial growth on the straw-based substrate treated at 75 and 121 °C. However, *L. boryana* strains showed positive correlations only on the MEA medium. Then the mycelial area increase on YMEA+WSLD for the former species is correlated with increase of the area of the mycelial growth in wheat straw but for the latter species the mycelial area increase on YMEA+WSLD is correlated with the reduction of the mycelial area on wheat straw (Table 4). Thus, it should be considered that our tests of mycelial growth on YMEA+WSLD (for *L. edodes* strains) and MEA (for both species) are good indicators of the potential of these strains to grow upon and exploit the resources of a wheat straw based cultivation substrate. By undertaking measurements of mycelial diameter in C2, the reliability of these tests was improved, primarily because the phenomenon of physiological adaptation to the culture medium, a time dependent factor, was taken into account. Thus, tests of C2 mycelial growth on the YMEA+WSLD culture medium, for *L. edodes* strains, can be considered as a selection criterion for the rate of initial growth on the substrate derived from wheat straw. The speed of initial growth is one of the principal elements used to adapt shiitake strains to cultivation on straw. In addition, this system of measuring mycelial growth on two successive cultures allows to estimate the speed of adaptation to the medium, especially when differential responses are related to the presence of specific compounds in the medium. The results of this work showed differences between species and strain adaptation ability, this information is useful for strain selection. However further experiments should be carried out in order to test selected strains and correlate adaptation ability to feasibility of improving yield.

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