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Influence of the culture medium and pH on the growth of saprobic and ectomycorrhizal mushroom mycelia

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***Aim*. This paper reports the influence of culture medium and pH in the radial growth and growth rate of saprobic (*Leucopaxillus giganteus*) and ectomycorrhizal (*Lactarius deliciosus* and *Suillus luteus*) edible mushrooms mycelium. *Leucopaxillus* species are used in chemical industry for extraction of clitocybin antibiotic and *Lactarius deliciosus* and *S. luteus* are highly consumed and commercialized in Trás-os-Montes region (Northeast of Portugal).**

***Methods*. Two different solid culture media (Melin-Norkans, MMN and Potato Dextrose Agar, PDA) and two pH values (5 and 6) were tested in order to achieve the best conditions to mycelium growth.**

***Results*. The pH had no influence in its morphological characteristics, while the culture medium only affected *S. luteus* mycelium. For all the tested conditions, the growth rate of *Leucopaxillus giganteus* mycelium was significantly higher ($P<0.05$) than the growth rate of ectomycorrhizal mushrooms mycelium.**

***Conclusion*. The pH had a significantly influence in the growth of *Leucopaxillus giganteus* and *S. luteus* when PDA medium was used, and MMN proved to be a better culture medium for *Lactarius deliciosus* and *S. luteus* mycelia growth.**

Key words: **Edible mushrooms - Mycorrhiza - Saprobic - Mycelium growth.**

Mushrooms have become attractive as a functional food and as source for the development of drugs and nutraceuticals,[1, 2] namely for antioxidant[3-9] and antimicrobial compounds.[10] In addition to dried mushrooms, alternative or substitute mushroom products are mycelia that could also be used as food and food-flavouring material, or in the formulation of nutraceuticals and functional foods. The nutritional value and taste components of some mushroom mycelia have also been studied.[11]

The northeast of Portugal, due to their climatic conditions and flora diversity, is one of the European regions with higher wild edible mushrooms diversity, some of them with great gastronomic relevance. *Lactarius deliciosus* (L.) Gray, *Suillus luteus* (L.) Gray and *Leucopaxillus giganteus* (Sowerby) Singer are important local edible species for their high harvesting and consumption in the rural population, and also due to their commercialization and economic value in the international market, as Spain, France and Italy.[12, 13] These *Basidiomycete* fungi belong to the order *Russulales*, *Boletales* and *Agaricales*, and to the families *Russulaceae*, *Boletaceae* and *Tricholomataceae*, respectively. Saprobic *Leucopaxillus* species have been reported for their medicinal activity, being used in chemical industry for extraction of clitocybin antibiotic. The other mushrooms are ectomycorrhizal (associations between fungi and some plant roots).

The growth of saprobic edible mushroom with its fruiting body as the most common edible form is a lengthy and complex process involving the use of

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TABLE I.—*Mean radial growth (cm) of Lactarius deliciosus, Suillus luteus and Leucopaxillus giganteus cultures, in the presence of different nutritive culture medium (MMN or PDA) and pH values (5 or 6), along time of inoculation.*

Days after inoculation	Radial growth[a] (cm)			
	MMN pH 5	MMN pH 6	PDA pH 5	PDA pH 6
Lactarius deliciosus				
7	0	0	0	0
14	0.28±0.07 a	0.25±0.08 a	0.16±0.09 b	0 c
21	0.51±0.07 a	0.47±0.07 a	0.36±0.11 b	0.18±0.07 c
28	0.80±0.09 a	0.75±0.11 a	0.55±0.16 b	0.18±0.07 c
36	1.13±0.14 a	1.29±0.15 a	0.74±0.17 b	0.23±0.12 c
43	1.59±0.22 a	1.45±0.24 a	0.94±0.17 b	0.38±0.09c
51	2.03±0.34 a	2.05±0.42 a	1.11±0.24 b	0.48±0.10 c
58	2.55±0.42 a	2.61±0.52 a	1.13±0.18 b	0.55±0.13 c
64	2.92±0.44 a	2.94±0.52 a	1.16±0.22 b	0.62±0.12 c
72	3.36±0.41 a	3.40±0.49 a	1.23±0.22 b	0.80±0.16 c
80	3.67±0.44 a	3.70±0.37 a	1.31±0.10 b	1.08±0.33 b
Suillus luteus				
7	0.06±0.12 ba	0.07±0.09 a	0 b	0 b
14	0.31±0.10 a	0.28±0.17 a	0.13±0.11 b	0.10±0.08 b
21	0.31±0.10 a	0.33±0.20 a	0.13±0.11 b	0.10±0.08 b
28	0.61±0.10 a	0.63±0.37 a	0.25±0.16 b	0.15±0.14 b
36	0.88±0.20 a	0.93±0.60 a	0.26±0.16 b	0.18±0.20 b
43	1.73±0.47 a	1.51±0.88 a	0.54±0.27 b	0.22±0.19 b
51	2.06±0.63 a	1.81±1.06 a	0.70±0.35 b	0.22±0.19 b
58	2.37±0.83 a	2.37±0.83 a	0.87±0.44 b	0.25±0.19 c
64	2.73±0.93 a	2.49±1.12 a	1.21±0.53 b	0.41±0.19 c
72	3.07±1.03 a	2.65±1.13 a	1.68±0.63 b	0.55±0.24 c
80	3.29±1.09 a	2.93±1.13 a	2.01±0.70 b	0.84±0.42 c
Leucopaxillus giganteus				
7	0.56±0.15 b	1.67±0.37 a	1.66±0.09 a	1.42±0.43 a
10	0.78±0.22 b	2.37±0.35 a	2.32±0.59 a	2.13±0.59 a
14	1.44±0.51 b	3.40±0.24 a	3.06±0.19 a	3.19±0.59 a
17	2.08±0.77 c	3.91±0.23 a	3.38±0.22 b	3.67±0.41 ba
21	2.95±0.93 b	3.97±0.23 a	3.71±0.26 a	3.92±0.29 a
24	3.46±0.85 b	4.01±0.19 a	3.81±0.21 ba	3.97±0.24 a

a) Each value is expressed as mean ± standard deviation (n=5). In each row different letters mean significant differences ($p < 0.05$).

solid compost or lignocellulosic waste, such as straw or cotton, followed by a long cultivation period.[14]

Ectomycorrhizal mushroom fruiting bodies could not be grown at all to data. Therefore, growing mushroom mycelium on a defined nutrient medium could be an alternative method to produce fungal biomas.[15]

In this study, we evaluated the influence of different culture media and pH values on the radial growth and growth rate of *Leucopaxillus giganteus*, *Lactarius deliciosus* and *S. luteus* mycelium. The mycelium growth was weekly followed during 80 days for ectomycorrhizal mushrooms and 24 days for the saprobic mushroom, and morphological characteristics were also evaluated for each tested conditions.

Materials and methods

Samples

Leucopaxillus giganteus (Sowerby) Singer was collected in grassland whereas *Lactarius deliciosus* (L.) Gray and *Suillus luteus* (L.) Gray were collected under living pine trees (*Pinus* sp.), in Bragança (northeast of Portugal), in autumn 2005. Taxonomic identification was made according to several authors [18-22] followed by liophylization (Ly-8-FM71 ULE, Snijders, Holland). Representative voucher specimens were deposited at the herbarium of Escola Superior Agrária of Instituto Politécnico de Bragança.

Isolation of the biological material

Mycelia of *Leucopaxillus giganteus*, *Lactarius deliciosus* and *S. luteus* were isolated from sporocarps, on agar Melin-Norkans (MMN) medium (NaCl 0.025 g/L; $(NH_4)_2HPO_4$ 0.25 g/L; KH_2PO_4 0.50 g/L; $FeCl_3$ 0.050 g/L; $CaCl_2$ 0.50 g/L; $MgSO_4.7H_2O$ 0.15 g/L; thiamine 0.10 g/L; casamino acids 1 g/L; malt extract 10 g/L; glucose 10 g/L; agar 20 g/L), following Brundrett *et al.*[23] The strains were maintained in Petri dishes (9 cm diameter) containing the same medium at 25ºC in the dark and subcultured every 2 weeks.

Effect of culture medium and pH on mycelia growth

The effect of culture medium and pH on mycelia growth of *Leucopaxillus giganteus*, *Lactarius deliciosus* and *S. luteus* was performed in Petri dishes (9 cm diameter) containing 10 mL of solid medium. The following different nutritive solid medium were tested: Melin-Norkans (MMN) medium pH 5 or 6, and Potato Dextrose Agar (PDA: potato 4 g/L; dextrose 20 g/L; agar 15 g/L) pH 5 or 6. Inoculation of Petri dishes was performed with hyphal plugs (created with a pipette tip, 5 mm diameter) of 2-week-old mycelia (one plug per plate). Five replicates for each medium were performed. The cultures were incubated at 25ºC in the dark. Fungal growth (colony radius) was measured weekly at 4 right angles after 80 days of inoculation. In the case of *Leucopaxillus giganteus* the growth was followed in smaller periods (3 days) until 24 days of inoculation.

Mycelia morphological description

The morphological description of mycelia growth in the different nutritive solid media was weekly performed. The parameters recorded were: colony texture and color, border appearance and color, reverse color, medium coloration, aerial growth, exudates and rifts.

Statistical analysis

Each experiment was carried out using 5 Petri dishes (4 colony radius values in each one) and the results are expressed as mean values and standard error. Differences among means were done by analysis of variance (ANOVA), using SAS v. 9.1.3, and averages were compared using Tukey test ($P < 0.05$).

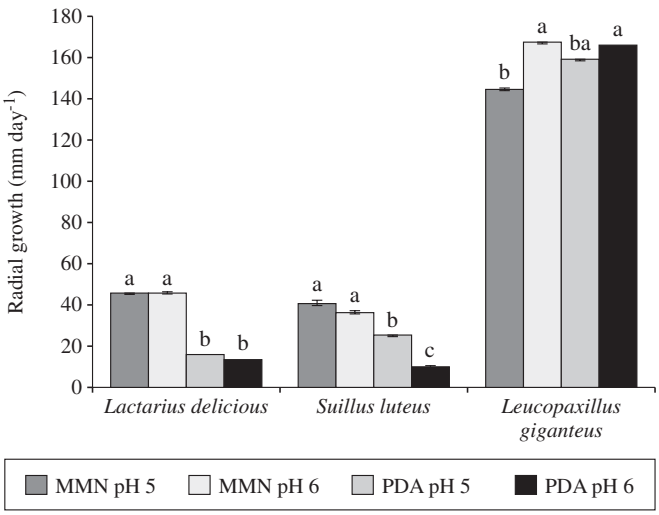

Figure 1.—Mean radial growth rate (mm day-1) of *Lactarius deliciosus*, *Suillus luteus* and *Leucopaxillus giganteus* cultures, in the presence of different nutritive culture medium (MMN or PDA) and pH values (5 or 6). Each value is expressed as mean ± standard error (n=5). Different letters above column groups indicate significantly different means at P<0.05.

Results

Effect of culture medium and pH on mycelia growth

The mycelium growth in different solid nutritive culture media (MMN or PDA) and at different pH values (5 or 6) is shown in Table I. In general, the mycelium growth varied either with the culture medium or pH medium. For *Lactarius deliciosus*, MMN proved to be a better culture medium than PDA. Although the pH had no influence on the mycelium growth using MMN medium, for PDA culture medium pH 5 was significantly (P<0.05) better than pH 6. The growth of *L. deliciosus* mycelium in all the tested culture conditions significantly (P<0.05) increased till the end of the experiment.

For *S. luteus*, the use of MMN culture medium instead of PDA significantly (P<0.05) increased the mycelium growth and once more, pH had no influence on the mycelium growth using this medium. In the case of PDA culture medium, pH 5 was only significantly (P<0.05) better than pH 6 between 58 and 80 days of growth. The growth of *S. luteus* mycelium in MMN medium significantly (P<0.05) increased during the whole experiment, while culture radius in PDA only increased after 58 days.

The growth of *L. giganteus* in PDA culture medium

TABLE II.—*Morphological description of L. deliciosus, and L. giganteus grown in different nutritive culture media and at different pH values.*

Species	Culture medium	pH	Mycelium texture	Mycelium color	Border	Border color	Reverse color	Aerial growth	Medium coloration	Exudates	Rifts
L. deliciosus	MMN	5.0	Wooly	Beige	Diffuse	White	Yellowish	-	-	-	-
		6.0	Wooly	Beige	Diffuse	White	Yellowish	-	-	-	-
	PDA	5.0	Wooly	Beige	Diffuse	White	Yellow/green	-	-	-	-
		6.0	Cottony	Beige	Clear	White	Yellow/green	-	-	-	-
S. luteus	MMN	5.0	Furry[a]	Whitish brown[b]	Clear	White	Brown	+	-	-	-
		6.0	Furry[a]	Whitish brown[b]	Clear	White	Brown	+	-	-	-
	PDA	5.0	Cottony	Brownish white[b]	Clear	White	Brownish/orange	+	+	-	-
		6.0	Cottony	Brownish white[b]	Clear	White	Brownish/orange	+	+	-	-
L. giganteus	MMN	5.0	Cottony	White	Diffuse	White	White	+	-	-	-
		6.0	Cottony	White	Diffuse	White	White	+	-	-	-
	PDA	5.0	Cottony	White	Diffuse	White	White	+	-	-	-
		6.0	Cottony	White	Diffuse	White	White	+	-	-	-

a) Cottony until 43 days of growth; b) white until 21 days of growth

at both pH and in MMN at pH 6 was very similar ($P>0.05$) until 14 days. After 17 days of growth differences were significant ($P<0.05$) and in the order MMN, pH 6 > PDA, pH 6 > PDA, pH 5. When MMN at pH 5 was used the mycelium growth was significantly ($P<0.05$) lower in comparison to pH 6. The growth of this mushroom mycelium only significantly increased ($P<0.05$) until 21 and 17 days of growth for pH 5 and 6, respectively. After these points and till the end of the experiments the radial growth did not increase.

After 24 days, *L. giganteus* (saprobic) mycelium had grown for all the Petri dish, while the growth of *L. deliciosus* and *S. luteus* (ectomycorrhizal) had to be followed until 80 days.

In Figure 1 we present the mycelia growth rate in different culture media and at different pH values. For all the tested conditions, the growth rate of *Leucopaxillus giganteus* mycelium (*e.g.* 166.90±0.19 mm/day in MMN6) was significantly higher ($P<0.05$) than the growth rate of ectomycorrhizal mushroom mycelium (46.22±0.37 mm/day for *Lactarius deliciosus* and 36.65±1.13 mm/day for *S. luteus*). The growth rate was very similar for *Lactarius deliciosus* and *S. luteus* being MMN the best culture medium.

Discussion

Mycelia morphological description

Morphological description of the mycelia grown in different culture media and at different pH values is presented in Table II. The pH had no influence on the mycelial morphological characteristics, while the culture medium only affected *S. luteus*.

L. deliciosus mycelium grown under the different conditions was beige and woolly, unless for PDA medium at pH 6 in which it became cottony. The border was white and diffuse changing to clear only in PDA 6 (Figure 2). The reverse in MMN and PDA culture media was yellowish and yellow/green, respectively. It was not observed any aerial growth, medium coloration, exudates or rifts.

S. luteus mycelium grown in MMN culture medium was whitish brown and furry, changing to brownish white and cottony in PDA culture medium. In both cases, the border was white and clear (Figure 2). The reverse was brown and brownish orange for MMN and PDA culture medium, respectively. It was observed aerial growth, and in the case of PDA culture the medium was also colored.

L. giganteus presented the same characteristics in both culture media and pH values (Figure 2). The mycelium was white and cottony, the border was white and diffuse, and the reverse was also white. In all the cases, it was possible to observe aerial growth.

Overall, the pH had no influence in mycelia morphological characteristics, and the culture medium only affected *S. luteus* mycelium characteristics. For all the tested conditions, the growth rate of *Leucopaxillus giganteus* mycelium was significantly higher than the growth rate of ectomycorrhizal mushrooms mycelium. For the latter (*Lactarius deliciosus* and *S. luteus*) MMN proved to be the better nutritive medium and it has been

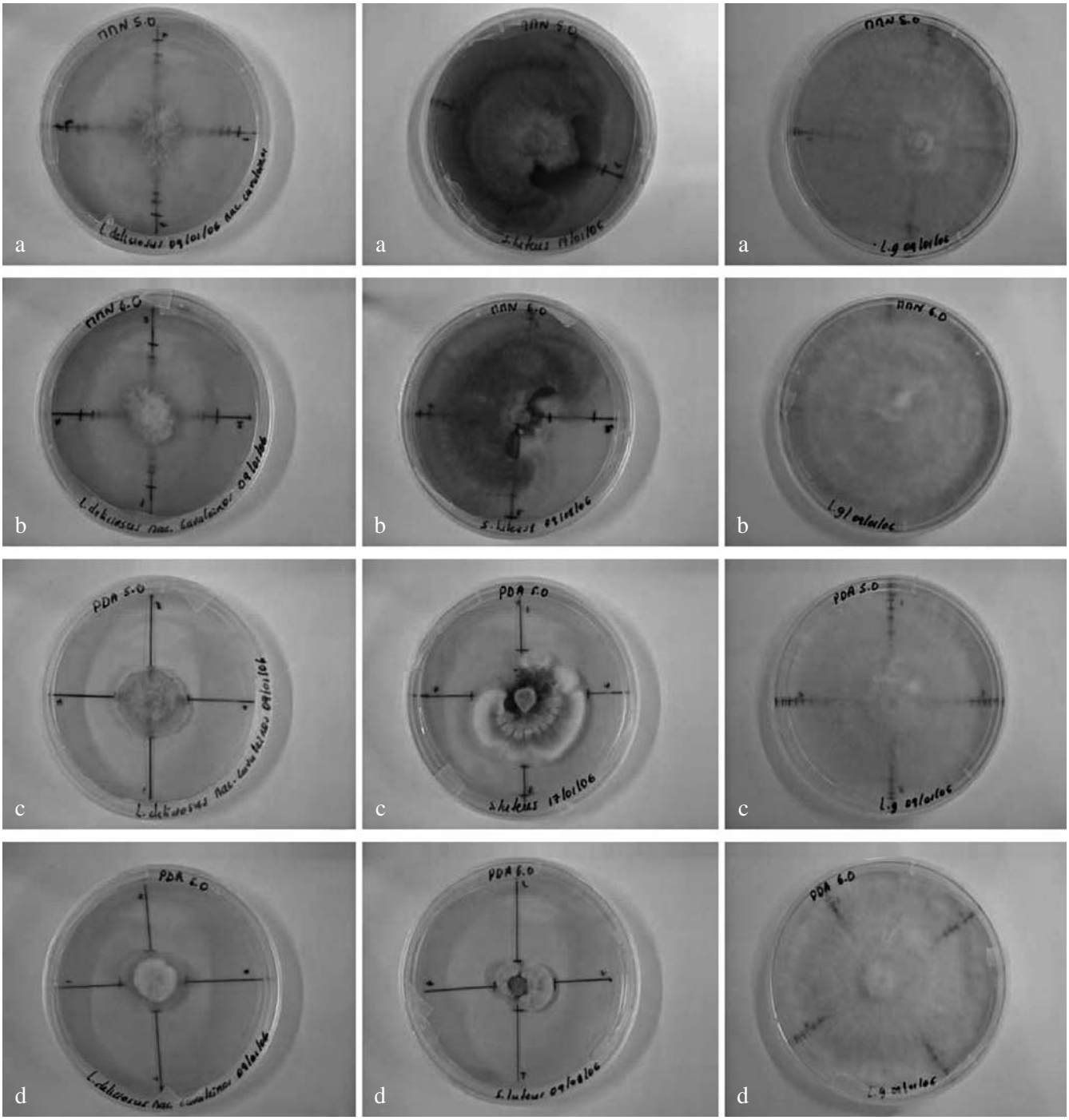

Figure 2.—Appearance of *Lactarius deliciosus, Suillus luteus* (after 80 days of growth) and *Leucopaxillus giganteus* (after 24 days of growth) colonies, in the presence of different nutritive culture medium and pH values after 80 days of growth: (a) MMN pH 5, (b) MMN pH 6, (c) PDA pH 5, (d) PDA pH 6.

referred as the most used medium and which usually offers the best results for ectomycorrhizal fungi.[24, 25] It is also known that pH culture medium influence the fungi growth. Although some studies reported considerable growth values between pH 3.2 and 6.5, the optimal pH ranges between 4.5 and 5.5. Fungi develop in optimum pH ranges which are related to enzymatic systems, essential vitamin entry in the cell, surface metabolic reactions and mineral capture. Some fungi, however, are able to adjust to the pH of the medium, optimising it for their better development.[26, 27] In our study the pH had a significantly influence in the growth of *Leucopaxillus giganteus* and *S. luteus* when PDA medium was used. Nevertheless, the results of the *in vitro* assays testing the pH effect on fungi growth should be carefully analysed since it may be influenced by the incubation time, nitrogen source and iron salts addition before or after the medium sterilization.[26]

The results obtained imply that the mycelium growth and appearance not only varies with the culture medium and/or pH value, but also changes with the mushroom species, being the saprobic mycelium growth rate significantly higher than the growth rate of ectomycorrhizal mushrooms mycelium.

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