In vitro mycelial behavior of native *Phellinus* strains

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Aceito para publicação em 03/06/2003

Resumo

No presente trabalho estudaram-se quatro culturas dicarióticas de espécies de *Phellinus* em diferentes meios e temperaturas. O comportamento do micélio foi testado em meios sólidos (crescimento radial e morfologia) e líquidos (produção de biomassa) em duas temperaturas 25 °C e 28 °C. As culturas de *P. punctatus* e *P. umbrinellus* cresceram mais rápido em meio ágar extrato de malte, enquanto tal comportamento em *P. flavomarginatus*, e *P. gilvus* foi em ágar aveia. Mudanças morfológicas foram observadas em *P. gilvus* e *P. punctatus* no meio ágar extrato de malte em 28 °C, ágar aveia a 25 °C e 28 °C e em *P. flavomarginatus* não foram observadas hifas esqueletais no meio ágar extrato de malte a 28 °C. Em meio líquido, a temperatura de 28 °C foi a melhor para produção de biomassa para *P. punctatus* (caldo de aveia) e *P. umbrinellus* (caldos batata e aveia). *Phellinus flavomarginatus* produziu, em ambas temperaturas testadas, mais biomassa em caldo batata. Por outro lado, *P. gilvus* cresceu melhor a 25 °C e em caldo batata.

Unitermos: Basidiomycetes, Hymenochaetaceae, biomassa, crescimento radial, podridão branca

Abstract

Four native dicaryotic strains of *Phellinus* species were examined under different media and temperature. Mycelial behavior in solid (radial growth and morphology) and liquid (biomass production) media (25°C and 28 °C) was tested. The solid media for the best radial growth were for *P. flavomarginatus* and *P. gilvus* oat agar. *Phellinus punctatus* and *P. umbrinellus* developed faster on malt extract agar, although, *P. umbrinellus* presented an almost similar growth on potato dextrose agar media. Morphological changes were observed in *P.gilvus* and *P. punctatus* strains on malt extract agar at 28 °C and oat agar at 25 °C and 28°C. *Phellinus flavomarginatus* did not show any skeletal hyphae on malt extract agar at 28 °C. *P. umbrinellus* did not present morphological modifications for any of the conditions tested. In liquid media, the best biomass production at 28 °C was for *P. punctatus* (oat broth) and *P. umbrinellus* (potato broth and oat broth). *Phellinus flavomarginatus* (potato broth) presented best biomass production at both temperatures. In contrast, *P. gilvus* (potato broth) grew better at 25°.

Key words: Basidiomycetes, biomass, radial growth, white rot, Hymenochaetaceae

Introduction

The genus *Phellinus* Quélet (Hymenochaetaceae, Basidiomycetes) includes about 154 species of wood white-rot fungi which are capable of degrading lignin, cellulose and hemicellulose (Larsen and Cobb-Poulle, 1990). Thus, it plays a significant role in wood decay due this ability, mainly in lignin

degradation (Blanchette, 1991; Worrall et al., 1997). This natural capacity has been used in several biotechnological processes, such as pulp biobleaching, enzyme production and treatment of pollutants (Addleman and Archibald, 1993; Reid and Paice, 1994; Reddy, 1995; Carvalho et al., 1998).

The efficiency and the preferences of an organism for growth a certain substrate has been studied for many years, but only in a few species of Basidiomycetes. *Phellinus*, on Santa Catarina Island in southern Brazil, is represented by eleven species (Loguercio-Leite and Wright, 1995). Strains obtained from fungi collections on Santa Catarina Island, *Phellinus flavomarginatus*, *P.umbrinellus*, *P. gilvus* and *P. punctatus* were selected for our study. Conditions for the best mycelial growth and temperature (25° or 28°C) using four native strains were investigated. Such knowledge about mycelial growth is useful in relation to both experimental and industrial uses, especially in bioreactors, where the faster growth is a distinct advantage in the generate of biomass (Salmones et al., 1990).

Material and Methods

Fungi and inoculation. Native strains of *Phellinus flavomarginatus* (12K), *P. gilvus* (546), *P. punctatus* (674) and *P. umbrinellus* (828) were obtained from the Culture Collection of the Mycology Laboratory in the Botanical Department, Federal University of Santa Catarina, Brazil. Inoculation was carried out with one 5-mm plug inoculum, obtained from 7-day-old cultures grown on malt extract agar at 28° C.

Media. Malt extract agar (MEA: 48 g/L - Merck), potato dextrose agar (PDA: 39 g/L - Sigma), and oat agar (OA: 72.5 g/L - Sigma) were used. Malt extract broth (MEB, DIFCO), potato dextrose broth (PDB, SIGMA), or oat broth (OB), 20mL, in 50mL Erlenmeyer flasks, were employed. The oat broth medium was made with 200g commercial oat flakes boiled in 1L water.

Radial growth, morphology and biomass production. The radius of the colony was measured for each strain after two weeks' growth, on 8 plates for each medium and temperature. The microstructures were observed under a light microscope at 1000X. The biomass was measured by vacuum filtration of the liquid culture. After filtration, the filters with mycelia were dried at 80°C for 48 hours.

Results and Discussion

The mycelial behaviors varied according to the strain's species, the cultural media and the temperatures (Table 1). The optimum medium and temperature for radial growth was observed on oat agar (AO) for *P. gilvus* (28 °C) and *P. flavomarginatus* (25° and 28°C). For *P. punctatus* and *P. umbrinellus*, the best medium was malt extract agar (MEA). Our data showed that radial growth was improved in relation to rising temperature, a result which was similar to that obtained by Salmones et al. (1990) in studies on *Laetiporus sulphureus*. All strains showed different radial growth on MEA and PDA, differing from Lonergan et al. (1994) who detected similar patterns of radial growth in *Phanerochaete chrysosporium* on MEA and PDA.

Morphological changes were observed in *P. gilvus* and *P. punctatus* strains (MEA 28°C, OA 25°C and 28°C) where chlamidospores (a thick-walled resting spore), usually present in MEA at 25°C (Neves, 1998), were not produced; *P. flavomarginatus* did not show any skeletal hyphae in MEA (28°C). *P. umbrinellus* did not show morphological modifications for any of the conditions tested.

There is no relationship between the largest radial growth and the biomass production in the media and temperatures tested. The biomass produced at 25°C (PDB) was more significant than at 28°C, except for *P. punctatus* and *P. umbrinellus* in OA (Figures 1 and 2), differing from *Phanerochaete chrysosporium* where the increase in temperature did not affect biomass production (Vyas et al. 1994).

TABLE 1 – The radial growth and biomass production of *Phellinus flavomarginatus*, *P. gilvus*, *P. punctatus* and *P. umbrinellus* strains, at 25° and 28°C, in different media.

		P. flavomarginatus		*P.gilvus*		*P. punctatus*		*P. umbrinellus*	
Media*	Temp. (°C)	Radial Growth (cm)	Biomass (g dry wt/mL)	Radial growth (cm)	Biomass (g dry wt/mL)	Radial growth (cm)	Biomass (g dry wt/mL)	Radial growth (cm)	Biomass (g dry wt/mL)
ME	25	6.2± 0.4	1.85± 0.30	6.1± 0.2	0.78± 0.67	4.5± 0.2	1.51± 0.39	4.7± 0.3	0.67± 0.12
	28	6.3± 0.4	1.73± 0.37	5.3± 0.5	0.52± 0.31	4.9± 0.2	1.26± 0.82	6.7± 0.3	0.90± 0.14
PD	25	4.2± 0.7	8.19± 0.68	5.4± 0.3	12.09± 0.84	3.9± 0.4	0.63± 0.21	4.9± 0.3	4.78± 1.70
	28	4.7± 0.2	7.01± 0.86	4.6± 0.4	6.02± 1.72	4.2± 0.7	1.89± 0.65	6.5± 0.5	3.89± 0.59
O	25	8.1± 0.2	2.86 ±0.31	7.5± 0.3	2.18 ±0.31	3.7± 0,2	0.63± 0.43	5.0± 0.2	0.63± 0.21
	28	8.3± 0.8	4.03± 0.43	9.0± 0.0	2.48± 0.39	3.5± 0.2	4.03± 0.21	6.0± 0.2	4.71±0.79

*ME= malt extract, PD= potato dextrose and O= oat, for solid (agar) and liquid media (broth), radial growth and biomass production, respectively.

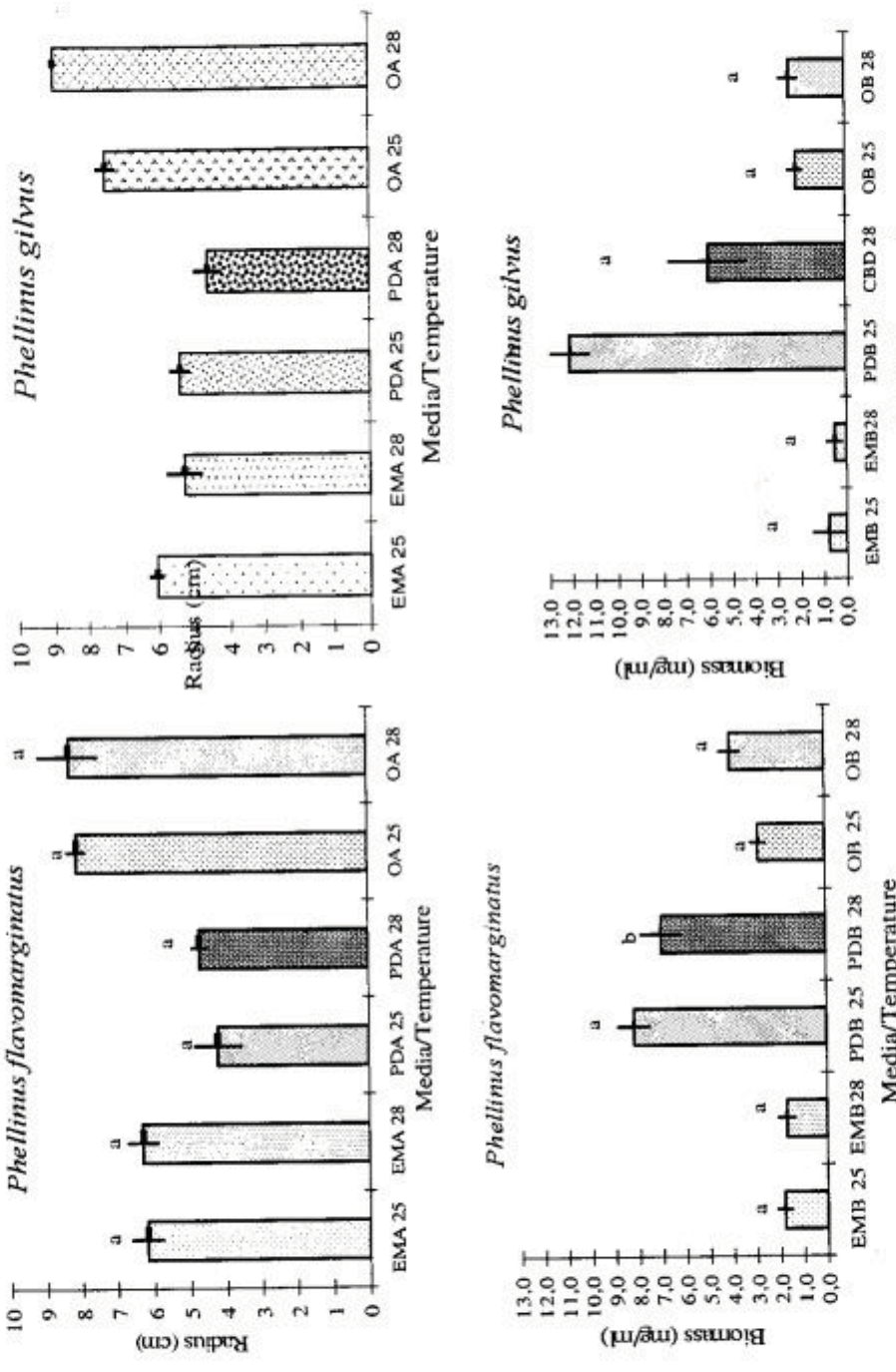

FIGURE 1: Radial growth and biomass production in *Phellinus flavomarginatus* and *P. gilvus* strains. Treatments not sharing letters are significantly different (< 0.05) from each other.

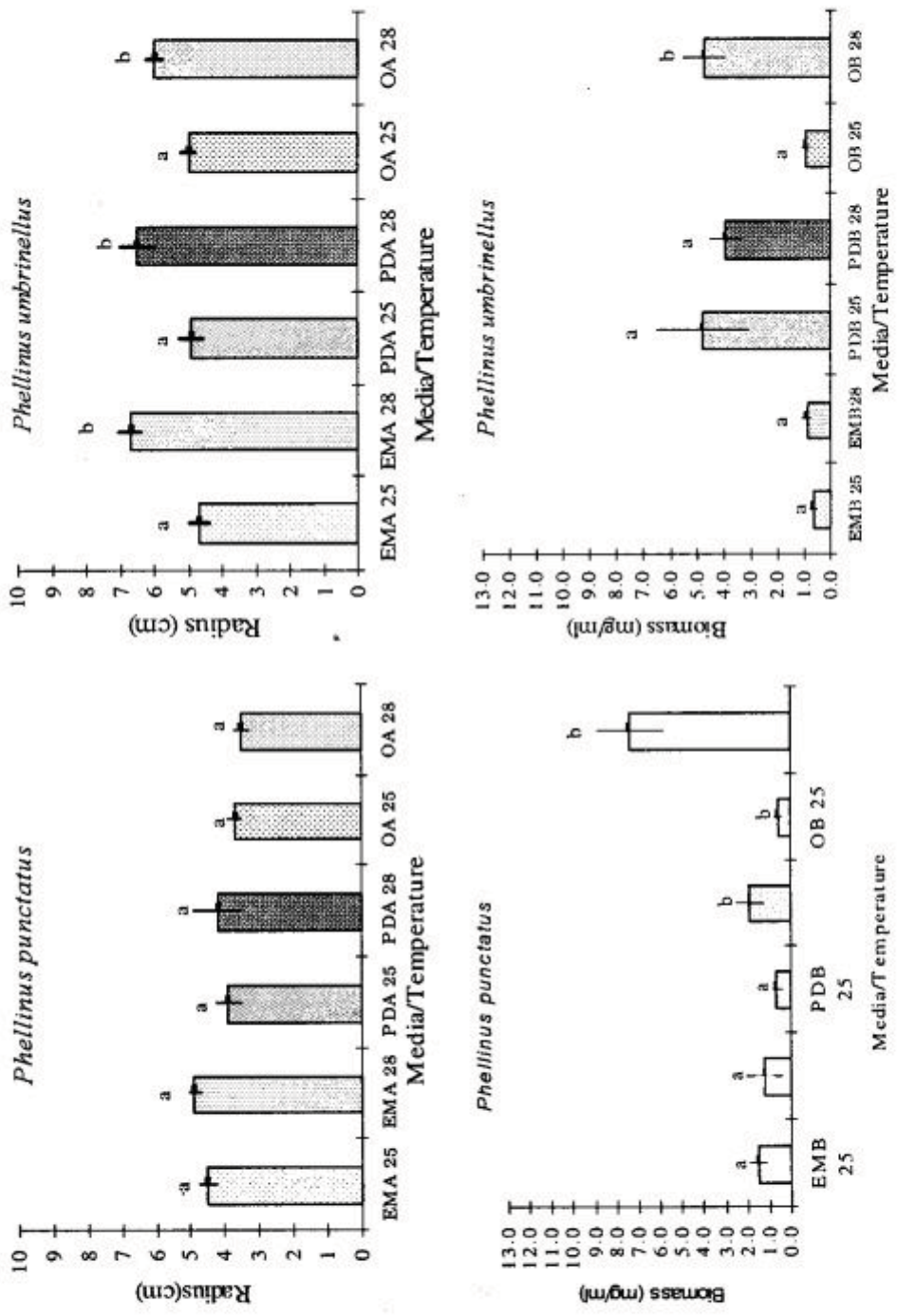

FIGURE 2: Radial growth and biomass production in *Phellinus punctatus* and *P. umbrinellus* strains. Treatments not sharing letters are significantly different (< 0.05) from each other.

Media and/or temperatures play an important role in the mycelial behavior (radial growth and biomass) of *Phellinus* strains. Morphological changes in *P. gilvus*, *P. punctatus* and *P. flavomarginatus* could be used as a helpful tool to show temperature and media alteration. The results are important to the extension of knowledge about fungal behavior, especially for applied studies; since the standardization of production mycelia in industrial applications must consider specific media and temperatures for each species to be used.

Acknowledgements

We are grateful to MSc Maria Alice Neves, Dr. Terezinha Paulilo (UFSC, SC, Brazil), and Dr. Elisa Esposito (UMC, SP, Brazil) for their invaluable criticism.

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