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Studies on Mycelial Growth and Biomass Production of Eight Lentinoid and Pleurotoid Species of Wild Edible Mushrooms from Brazil

Marina Pires Corrêa-Santos[1,2]
https://orcid.org/0000-0002-4329-9861

Eduardo Pereira Cabral Gomes *(in memoriam)*[2]
https://orcid.org/0000-0002-3537-2977

Mariana de Paula Drewinski[1,2]
https://orcid.org/0000-0002-7299-8477

Vera Maria Valle Vitali[3]
https://orcid.org/0000-0003-3628-4642

Leonardo Pimentel da Silva Pena[1]
https://orcid.org/0009-0001-2994-9506

Nelson Menolli Junior[1,2*]
https://orcid.org/0000-0002-1841-8179

Cristiano Coelho do Nascimento[1,2]
https://orcid.org/0000-0001-9573-1962

[1]Instituto Federal de Educação, Ciência e Tecnologia de São Paulo, *Campus* São Paulo, Departamento de Ciências da Natureza e Matemática, Subárea de Biologia, IFungiLab, São Paulo, SP, Brasil; [2]Instituto de Pesquisas Ambientais, Núcleo de Pós-graduação Stricto Sensu, Pós-graduação em Biodiversidade Vegetal e Meio Ambiente, São Paulo, SP, Brasil; [3]Instituto de Pesquisas Ambientais, Núcleo de Conservação da Biodiversidade, São Paulo, SP, Brasil.

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[*]Correspondence: menollijr@yahoo.com.br (N.M.J.).

HIGHLIGHTS

- First tests of growth and biomass for *Lentinus concavus* and *Panus* cf. *tephroleucus.*
- Physiological knowledge of mycelial development at high temperatures.
- Temperature study of strains from three Brazilian biomes: Pantanal, Atlantic Forest and Amazon.

ABSTRACT: Among the 22,000 known species of mushrooms, around 2,000 are edible and only 100 species are cultivated worldwide. Species occurring naturally in local habitats may be very promising for cultivation studies to introduce new edible species in the local mushroom market. The objective of this study was to evaluate the conditions of mycelial growth and biomass production of Brazilian wild isolates of edible mushrooms for future domestication and cultivation studies. The wild mushrooms were collected, obtained or recovered and identified the pure cultures, and evaluated the mycelial growth and biomass production in Potatoes Dextrose Agar medium of eight lentinoid and pleurotoid species at four to five different temperatures: 20°C, 25°C, 30°C, 35°C, 40°C, 45°C. Nine isolates of *Lentinus berteroi*, one of *Lentinus concavus*, five of *Lentinus crinitus*, three of *Panus strigellus*, one of *Panus* cf. *tephroleucus*, six of *Pleurotus albidus*, seven of *Pleurotus djamor*, and one of *Pleurotus pulmonarius* were identified and evaluated. The minimum colonization period (in days) on the Petri dish and the better temperature of both mycelial growth and biomass production for each species were as follows: three days and 35°C for *L. berteroi,* three days and 35-40°C for *L. crinitus*, four days and 35°C for *L. concavus* and *P. strigellus*, five days and 30-35°C for *Panus* cf. *tephroleucus*, six days and 25-30°C for *Pl. albidus* and *Pl. djamor*, and seven days and 30°C for *Pl. pulmonarius*. Thus, this study provides data on species selection and mycelial growth of wild isolates for further research on domestication and cultivation of edible mushrooms.

Keywords: biomes; Brazil; cultivation; domestication; temperature.

INTRODUCTION

A recent estimate about the number of fungi in the world indicates that there are approximately 1.5-6.3 million species, with a best estimate at 2.5 million [1], although approximately 155 thousand species are known [2,3]. For mushroom-forming fungi, it is estimated the existence of 22 thousand known species [4], of which 2,189 are edible [5] and represent around 10% of all known mushroom species.

In Brazil, the commercial edible mushrooms more known, cultivated, and consumed are *Agaricus bisporus* (J.E. Lange) Pilát, *Lentinula edodes* (Berk.) Pegler, and the species of the genus *Pleurotus* (Fr.) P. Kumm [6,7]. All of these are species native from countries with temperate climate conditions, which increases the costs of production in tropical countries [8]. This financial difficulty of production tends to change with the knowledge of species that occur naturally in local areas of tropical climate conditions. Thus, to know the edible mushroom species occurring in Brazil, Drewinski and coauthors [9] compiled data from bibliographies, public repositories, and new records, reaching a list with more than 400 species records to the country, of which about 80 species represent consistent records based on molecular data and/or Brazilian nomenclatural types.

The knowledge on wild edible mushrooms from Brazil was mainly obtained directly from indigenous communities [10,11,12,13,14,15,16] or from compilations of ethnomycological studies [17,18,19], which brought together data on collecting and consume of 45 mushrooms species from twelve ethnic groups from the Amazon.

Despite the considerable diversity of wild edible mushroom species in Brazil, little is known about the physiology of mycelial growth of wild isolates from local forests. Vargas-Isla and Ishikawa [8] studied an isolate of *Panus strigellus* (Berk.) Chardón & Toro from the Brazilian Amazon and concluded that the temperature of 35°C was the most efficient in relation to mycelial growth and biomass production. Negrão and coauthors [20] studied the mycelial growth of *Lentinus berteroi* (Fr.) Fr. from the southeast region of the state of São Paulo, Brazil, and concluded that at high temperatures, around 30°C, the *L. berteroi* isolate studied degrades the substrate more quickly. Drewinski and coauthors [21] studied wild isolates of *Auricularia fuscosuccinea* (Mont.) Henn. and *Laetiporus gilbertsonii* Burds. from fragments of the Atlantic Forest in Southern and Southeastern Brazil and concluded that at a temperature of 30°C the mycelial growth was faster and there was greater production of mycelial biomass. The temperatures evidenced in these studies are those that usually occur in summer and spring in Brazil.

Understanding physiological preferences is fundamental as part of the domestication process. Albertó [22] summarized the process of domestication of wild mushrooms into some stages, including: (1) selection of the mushroom species; (2) determination of optimal conditions for mycelial growth; (3) inoculum development; (4) substrate selection; (5) incubation period; (6) production condition; (7) harvest, among others. Here, this study was focused on the first two processes related to species selection and mycelial growth. Zervakis and coauthors [23] stated that during the mycelial colonization stage, the effect of environmental parameters plays an essential role in mycelial growth and contributes significantly to the entire cultivation process. They [23] also stated that denser mycelium can be attributed to favorable conditions and the exploitation of nutritional resources provided by the environment.

Among the 184 genera with edible mushroom species listed by Drewinski and coauthors [9] with records of occurrence in Brazil, we highlight for study in this work three genera of lentinoid and pleurotoid mushrooms: *Lentinus* Fr., *Panus* Fr., and *Pleurotus*. In this study, we evaluate the effect of temperature for the *in vitro* mycelial growth and biomass production of 33 wild isolates of eight edible mushroom species from the Brazilian forests: *Lentinus berteroi*, *L. crinitus* (L.) Fr., *L. concavus* (Berk.) Corner, *Panus strigellus*, *Panus* cf. *tephroleucus* (Mont.) T.W. May & A.E. Wood, *Pleurotus albidus* (Berk.) Pegler, *Pl. djamor* (Rumph. ex Fr.) Boedijn, and *Pl. pulmonarius* (Fr.) Quél.

MATERIAL AND METHODS

Sampling

A total of thirteen isolates were obtained from fresh mushrooms collected in three Brazilian states (Mato Grosso do Sul, Rio de Janeiro, and São Paulo) covering the Atlantic Forest and the Pantanal biomes (Figure 1, Table 1). Additionally, 20 isolates from the Brazilian states of Amazonas, Rio de Janeiro, and São Paulo have been recovered from the culture collection (CCIBt) of the 'Instituto de Pesquisas Ambientais' (IPA), São Paulo, Brazil (Figure 1, Table 1). From the fresh basidiomata, fragments were removed from the context of

the pileus or stipe and then inoculated into Petri dishes containing sterile Potato Dextrose Agar (PDA) medium. Fragments of the pure mycelium from fresh basidiomata or those recovered from the CCIBt were then inoculated in Petri dishes with PDA culture medium and incubated at 25°C until complete mycelial growth. The collected wild basidiomata were dried in a food dehydrator (42°C) until complete dehydration and are deposited at the fungarium FIFUNGI (IFungiLab, at the Instituto Federal de Educação, Ciência e Tecnologia de São Paulo – IFSP, São Paulo Brazil) or at the herbarium SP at IPA. The pure isolates are deposited at the CCIBt (Table 1). This study is according to the Brazilian legislation on access to genetic biodiversity heritage and is registered in the 'Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado' (SisGen #A724370).

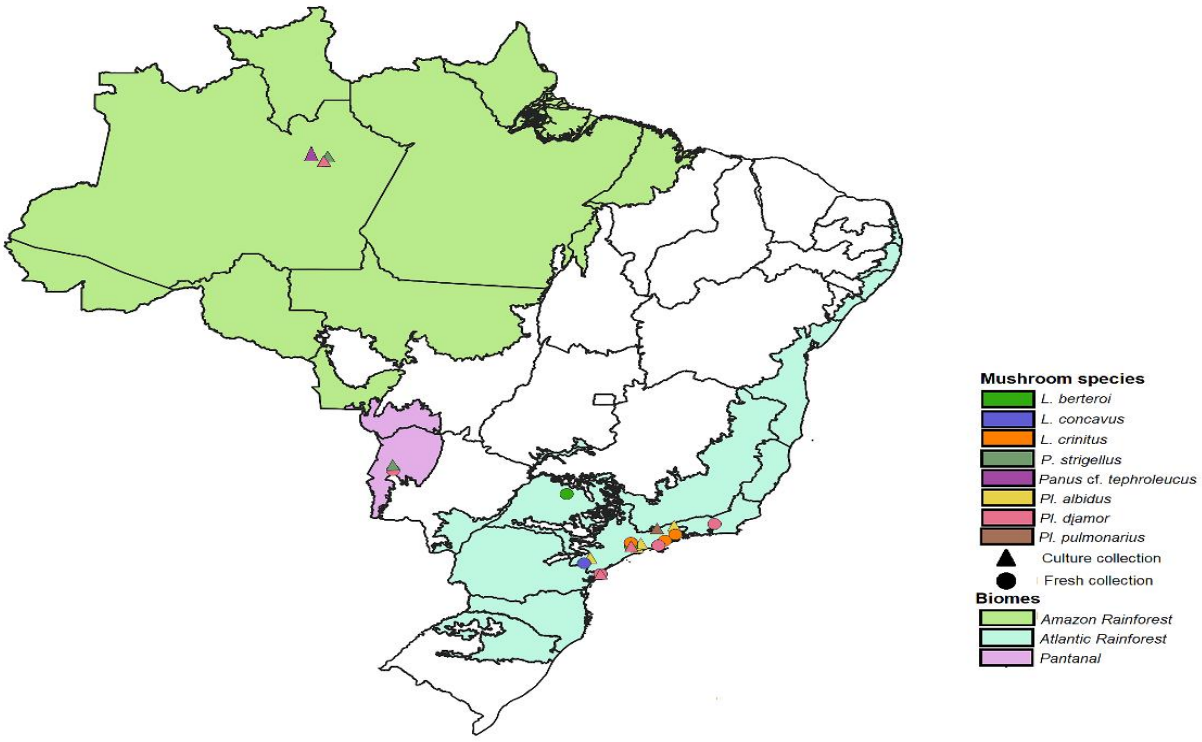

Figure 1. Map of the sampling areas of the studied collections and wild isolates.

Identification

Sequencing was conducted to confirm the identification of all pure cultures newly obtained and recovered. The DNA extraction was conducted from mycelium grown in liquid medium (Potato Broth Dextrose), which was distributed in Erlenmeyer flask (100 mL) and sterilized in an autoclave for 15 min at 121°C. The mycelium on liquid medium was obtained from five fragments of approximately 10 mm in diameter that were inoculated from the pure cultures on solid medium and then incubated for 7-12 days in a shaker at 26°C and 120 rpm [8]. After growth, the mycelium was filtered using a vacuum pump, stored in microtubes, and frozen for DNA extraction. The intergenic ribosomal region (ITS), considered barcoding for fungi, was amplified by polymerase chain reactions (PCR) with the primer pair ITS1-F and ITS4-R [24,25]. The amplified products were purified and sent for sequencing, using the same amplification primer pair, at the 'Centro de Estudos do Genoma Humano e Células-Tronco' at the 'Universidade de São Paulo' (USP), São Paulo, Brazil, or at Macrogen, Seoul, South Korea. The generated sequences were manually checked and edited, if necessary, in Geneious v.8.1 [26]. Then, the forward and reverse sequences were paired for obtaining the consensus sequence. The sequences obtained for each sample were compared to other sequences available in the GenBank database using the BLASTn similarity search method (http://www.ncbi.nlm.nih.gov/BLAST/). Thus, it was possible to certify the similarity of the obtained sequences with previously available sequences to determine or confirm the specific identity of the isolates. Additionally, Sousa-Guimarães and coauthors [27] and Menolli and coauthors [28] were considered for comparison and identification of the sequences generated from *Panus* and *Pleurotus* species, respectively. For species that did not have sequences in the database, a morphological study of the collected basidiomata was carried out to species identification based on Pegler [29].

Table 1. Data on the Brazilian wild isolates evaluated in this work.

Species	CCIBt accession	Collector number	Fungarium/ herbarium accession	GenBank accession	Locality in Brazil
Lentinus berteroi	CCIBt2868	-	-	PQ001141	São Paulo, Cananeia, PEIC
L. berteroi	CCIBt3005	-	-	PQ001142	São Paulo, São Paulo, IPA
L. berteroi	CCIBt3006	-	-	PQ001140	São Paulo, São Paulo, IPA
L. berteroi	CCIBt3008	-	-	PQ001144	São Paulo, São José do Rio Preto, CEENP
L. berteroi	CCIBt3009	-	-	PQ006019	São Paulo, São José do Rio Preto, CEENP
L. berteroi	CCIBt3369	-	-	PQ001143	São Paulo, São Paulo, IPA
L. berteroi	CCIBt4788	MPCS17	FIFUNGI457	PQ006018	São Paulo, Iporanga, PETAR
L. berteroi	CCIBt4796	MPCS88	FIFUNGI136	PQ001138	São Paulo, Caraguatatuba, PESM
L. berteroi	CCIBt4797	MPCS92	FIFUNGI151	PQ001139	São Paulo, Caraguatatuba, PESM
Lentinus concavus	CCIBt4792	NMJ430	FIFUNGI124	PQ001137	São Paulo, Iporanga, PETAR
Lentinus crinitus	CCIBt4795	MPCS74	FIFUNGI419	PQ001145	São Paulo, São Paulo, PEC
L. crinitus	CCIBt4790	MPCS96	FIFUNGI153	PP981001	São Paulo, Caraguatatuba, PESM
L. crinitus	CCIBt4791	MPD429	FIFUNGI22	PQ001146	São Paulo, São Luiz do Paraitinga, PESM
L. crinitus	CCIBt4794	NMJ357	FIFUNGI63	PQ001147	São Paulo, São Paulo, PEC
L. crinitus	CCIBt4793	NMJ456	-	PQ001148	Rio de Janeiro, Paraty
Panus strigellus	CCIBt3396	-	-	OR676909	Amazonas, Manaus
P. strigellus	CCIBt2499	-	-	OR676906	São Paulo, Ilha Solteira
P. strigellus	CCIBt3399	-	-	OR676908	Amazonas, Manaus
Panus cf. *tephroleucus*	CCIBt3398	-	SP446147	OR676911	Amazonas, Manaus
Pleurotus albidus	CCIBt2844	-	SP445804[a]	KF280336	São Paulo, Ribeirão Pires
Pl. albidus	CCIBt2930	-	SP445792[a]	KF280338	São Paulo, São Paulo
Pl. albidus	CCIBt2405	-	SP445809[a]	KF280339	São Paulo, Mogi das Cruzes
Pl. albidus	CCIBt4244	-	SP466412	PQ005732	Rio de Janeiro, Serra da Bocaina
Pl. albidus	CCIBt2731	-	SP445812[a]	KF280334	São Paulo, Ribeirão Grande
Pl. albidus	CCIBt2733	-	SP445801[a]	KF280332	São Paulo, Ribeirão Grande
Pleurotus djamor	CCIBt3030	-	-	PQ001149	Amazonas, Manaus
Pl. djamor	CCIBt2858	-	SP445798	KF280326	São Paulo, Cananeia, PEIC
Pl. djamor	CCIBt3978	-	SP445682	KF280324	São Paulo, São Paulo, IPA
Pl. djamor	-	MPD553	FIFUNGI30	PQ060187	Rio de Janeiro, Teresópolis, PARNASO
Pl. djamor	-	MPD241	SP528744	OQ621766	São Paulo, Cananeia, PEIC
Pl. djamor	CCIBt4719	MPD478	SP528776	OQ623833	Mato Grosso do Sul, Corumbá
Pl. djamor		MPD704	FIFUNGI147	PQ001150	São Paulo, Caraguatatuba, PESM
Pleurotus pulmonarius	CCIBt2963	-	SP445807[a]	KF280340	São Paulo, Campos do Jordão, PECJ

CCIBt: '*Coleção de Culturas de Algas, Cianobactérias e Fungos*' at the '*Instituto de Pesquisas Ambientais*'. CEENP: '*Estação Ecológica do Noroeste Paulista*'; IPA: '*Instituto de Pesquisas Ambientais*'. PARNASO: '*Parque Nacional da Serra dos Órgãos*'; PESM: '*Parque Estadual da Serra do Mar*'; PEC: '*Parque Estadual da Cantareira*'; PECJ: '*Parque Estadual de Campos do Jordão*'; PEIC: '*Parque Estadual da Ilha do Cardoso*'. [a] Basidiomata obtained from cultivation of CCIBt isolates.

Mycelial growth and biomass in culture media

The identified pure isolates were evaluated for mycelial growth and biomass production in PDA culture medium under different temperatures. After the preparation and solidification of the PDA culture medium (30 mL) in Petri dishes (90 mm), a fragment (10 mm diam.) of a previously colonized medium of each isolate was inoculated in the center of the Petri dishes. The plates were maintained in a biochemical oxygen demand (BOD) incubator at 20°C, 25°C, 30°C, and 35°C [30] for *P. strigellus, Panus* cf. *tephroleucus, Pl. albidus, Pl. djamor,* and *Pl. pulmonarius*, whilst *L. berteroi*, *L. crinitus*, and *L. concavus* were incubated at 25°C, 30°C, 35°C, 40°C and 45°C because they are species that develop on higher temperatures based on a pilot experiment [31].

To determine the minimum period of mycelium colonization (in days) of each species, i.e. when the complete colonization of the mycelium occurred throughout the Petri dish at a certain temperature, a pilot experiment in triplicate was performed, from which two perpendicular lines were drawn on the plates and followed up with daily measurements to determine the mycelial growth radius until complete colonization at different temperatures. Once the minimum period of mycelial colonization of each species was determined, the experiment was carried out in fifteen replicates for each isolate of each species at each temperature. To evaluate the dry mycelial mass, after growth until the minimum period of mycelial colonization of each species at one of the temperatures, the plates with culture medium were heated in the microwave for 20 s to melt the culture medium [8]. From the plate with the liquefied medium, the mycelium was filtered and washed with distilled water and then dehydrated at 75°C until constant weight to determine the dry mycelial biomass [8].

Statistical analyses

Mycelial growth and dry biomass data were assessed for normality using the Shapiro-Wilk test [32]. To verify the statistical difference, the data showing normal distribution were subjected to analysis of Variance – ANOVA and posterior to the Tukey test [32].

RESULTS

Lentinus berteroi

Nine isolates were tested of *L. berteroi* (Table 1) and the general growth average was higher ($p \leq 0.05$) for seven isolates (CCIBt2868, CCIBt3005, CCIBt3006, CCIBt3008, CCIBt3009, CCIBt3369, and CCIBt4788) that completed the growth in PDA culture medium in three days at 35°C (Figure 2A). The isolates CCIBt3006, CCIBt3009, CCIBt2868, and CCIBt3369 also showed a good mycelial growth at 30°C, and the average growth values of these isolates did not show a statistically significant difference between 30°C e 35°C ($p > 0.05$). About the dry mycelial mass (Figure 2B), the isolate CCIBt3009 had the best ($p \leq 0.05$) average biomass production at 35°C (333.61 mg ± 12.21).

Lentinus concavus

The isolate CCIBt4792 of *L. concavus* showed a better ($p \leq 0.05$) mycelial growth at 35°C (Figure 3A, 4A-E), having completed the growth in PDA culture medium in four days. Regarding the mycelial mass, on the fourth day of growth, the dry weight of biomass produced by the isolate CCIBt4792 was higher ($p \leq 0.05$) at 35°C (Figure 3B), with an average of 130.4 mg (± 9.91).

Lentinus crinitus

For the five tested isolates of *L. crinitus*, the highest general average of mycelial growth was at 35°C ($p \leq 0.05$), with emphasis on the isolates CCIBt4790, CCIBt4791, and CCIBt4794, which developed equally ($p \leq 0.05$) among them and better ($p \leq 0.05$) than the remaining isolates at 35°C (Figure 5A, 4F–J). The isolate CCIBt4790 performed equally better at 35°C (76.95 mm ± 1.73) and at 40°C (73.55 mm ± 3.24), with no statistical difference ($p > 0.05$) at both temperatures (Figure 5A). Regarding to the dry biomass (Figure 5B), the best result ($p \leq 0.05$) of the general average of all isolates was at 40°C (124.93 mg ± 10.63), with emphasis on the isolate CCIBt4790, which presented the highest ($p \leq 0.05$) dry mycelial mass value (157.1 ± 16.61 mg).

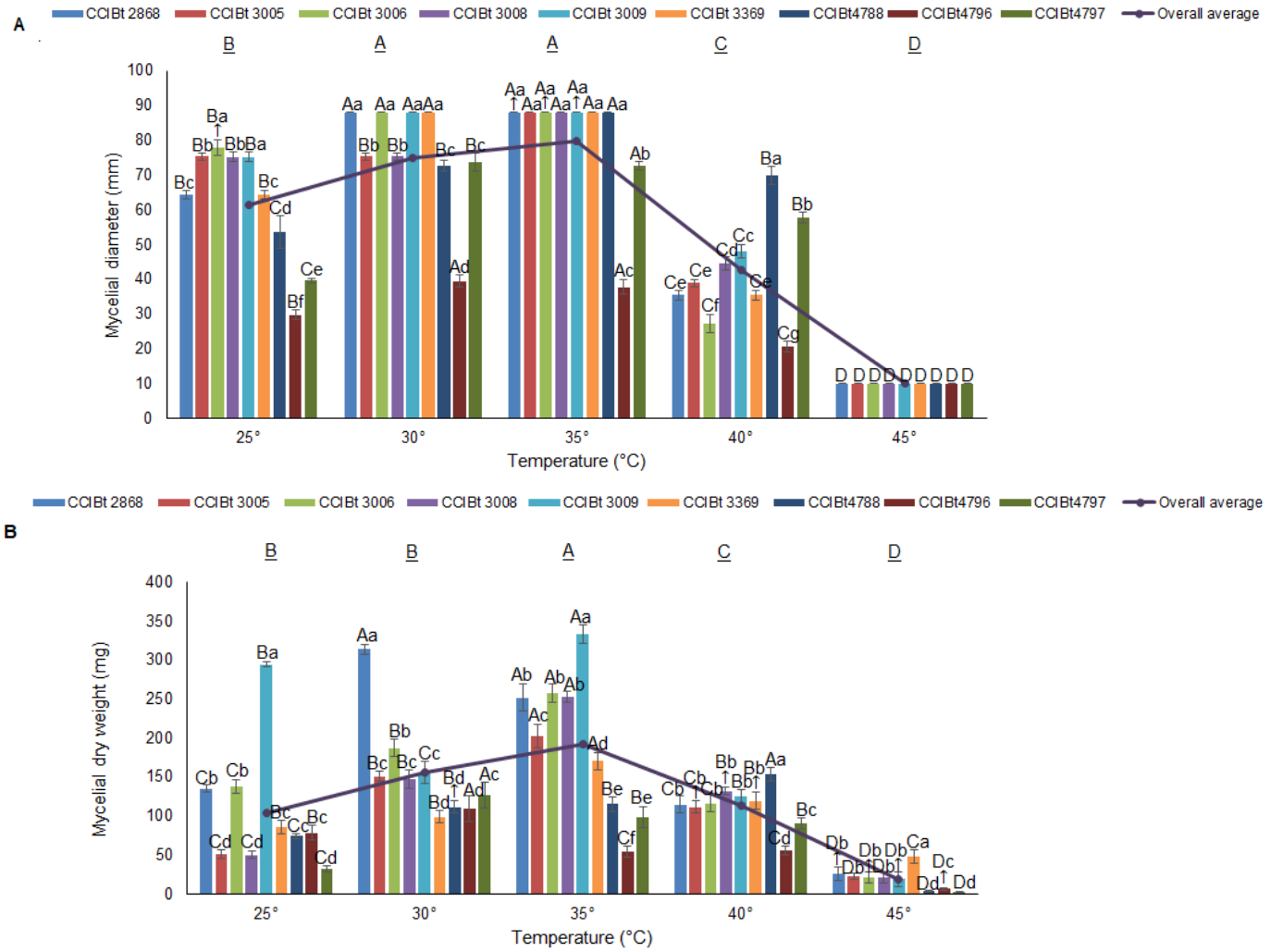

Figure 2. Mycelial growth of nine wild isolates of *Lentinus berteroi* on the third day of incubation in PDA culture medium at different temperatures. A. Mycelial diameter (mm). B. Mycelial dry weight (mg). Lowercase letters compare different isolates in the same temperature range. Capital letters compare the same isolate at different temperature ranges. Underlined capital letters compare the overall means of all isolates at different temperature ranges. Means followed by the same letter do not differ from each other using the Tukey test, at 5% probability. The absence of lowercase letters over the bars indicates a non-significant difference.

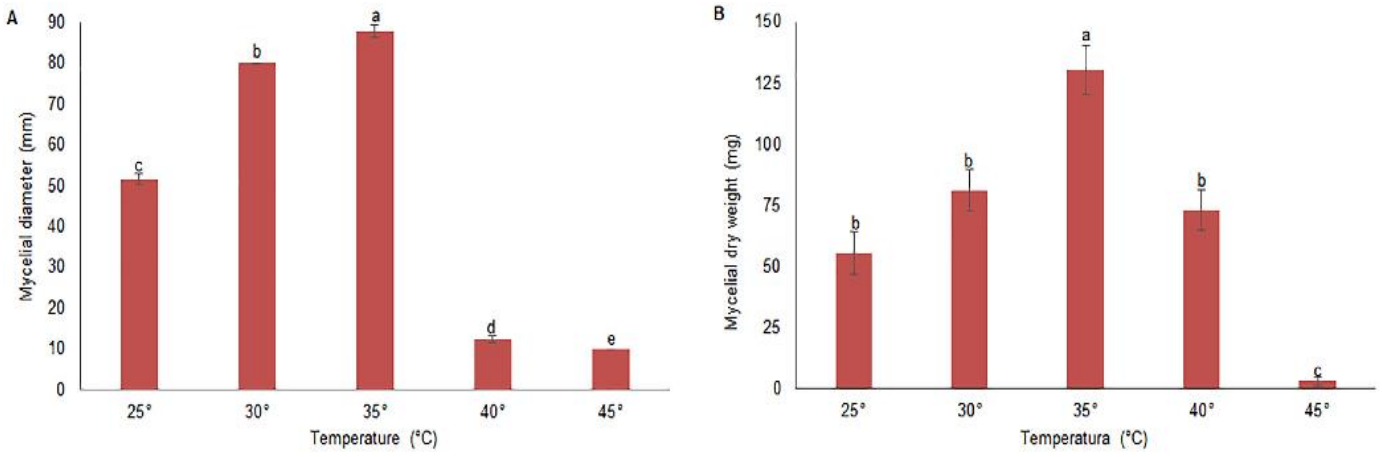

Figure 3. Mycelial growth of the wild isolate CCIBt4792 of *Lentinus concavus* on the fourth day of incubation in PDA culture medium at different temperatures. A. mycelial diameter (mm). B. Mycelial dry weight (mg). Lowercase letters compare different isolates in the isolate at different temperatures. Means followed by the same letter do not differ from each other using the Tukey test, at 5% probability.

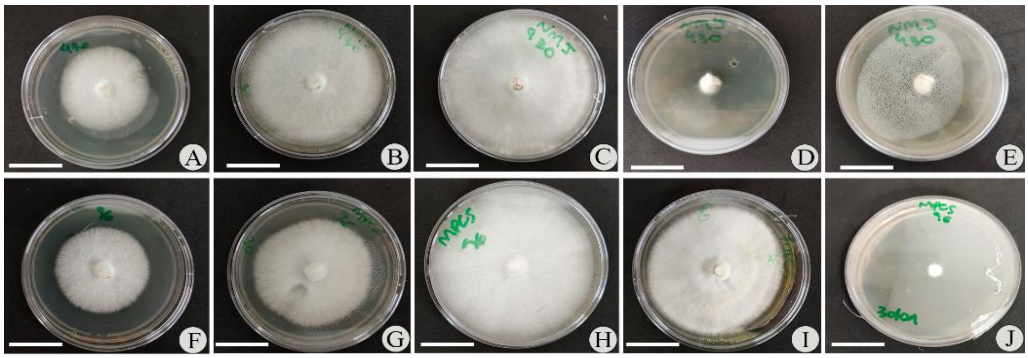

Figure 4. Mycelial growth of the wild isolate CCIBt4792 of *Lentinus concavus* in PDA medium on the fourth day. (A) 25°C. (B) 30°C. (C) 35°C. (D) 40°C. (E) 45°C. Mycelial growth of the wild isolate CCIBt4790 of *Lentinus crinitus* in PDA medium on the third day. (F) 25°C. (G) 30°C. (H) 35°C. (I) 40°C. (J) 45°C. Scale bars = 3 cm. Photos: Corrêa-Santos, M.P.

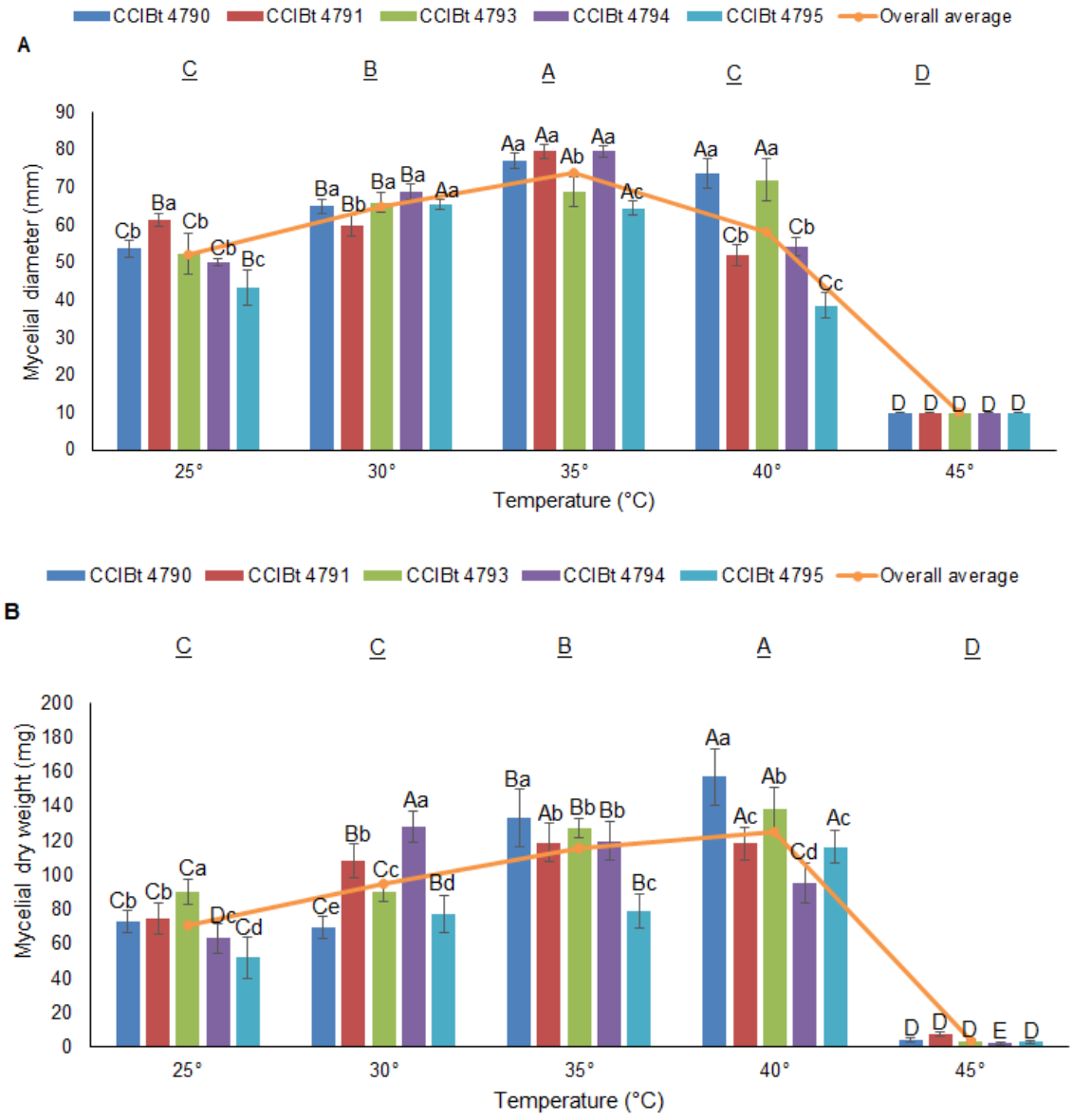

Figure 5. Mycelial growth of five wild isolates of *Lentinus crinitus* on the third day of incubation in PDA culture medium at different temperatures. A. Mycelial diameter (mm). B. Mycelial dry weight (mg). Lowercase letters compare different isolates in the same temperature range. Capital letters compare the same isolate at different temperature ranges. Underlined capital letters compare the overall means of all isolates at different temperature ranges. Means followed by the same letter do not differ from each other using the Tukey test, at 5% probability. The absence of lowercase letters above the bars indicates non-significant differences.

Panus strigellus

The general average of mycelial growth of the three tested isolates of *P. strigellus* was higher (p≤0.05) at 35°C (32.85 mm ± 1.28), with highlight to the isolate CCIBt2499 that completed the growth in PDA culture medium in four days and had statistically different mycelial geowth value (p≤0.05) than the other two isolates (Figure 6A). In relation to the mycelial mass on the fourth day, the dry weight averages for all isolates were highest (p≤0.05) at 35°C, with highlight to the isolate CCIBt2499 that had the highest (p≤0.05) biomass production at 35°C (Figure 6B).

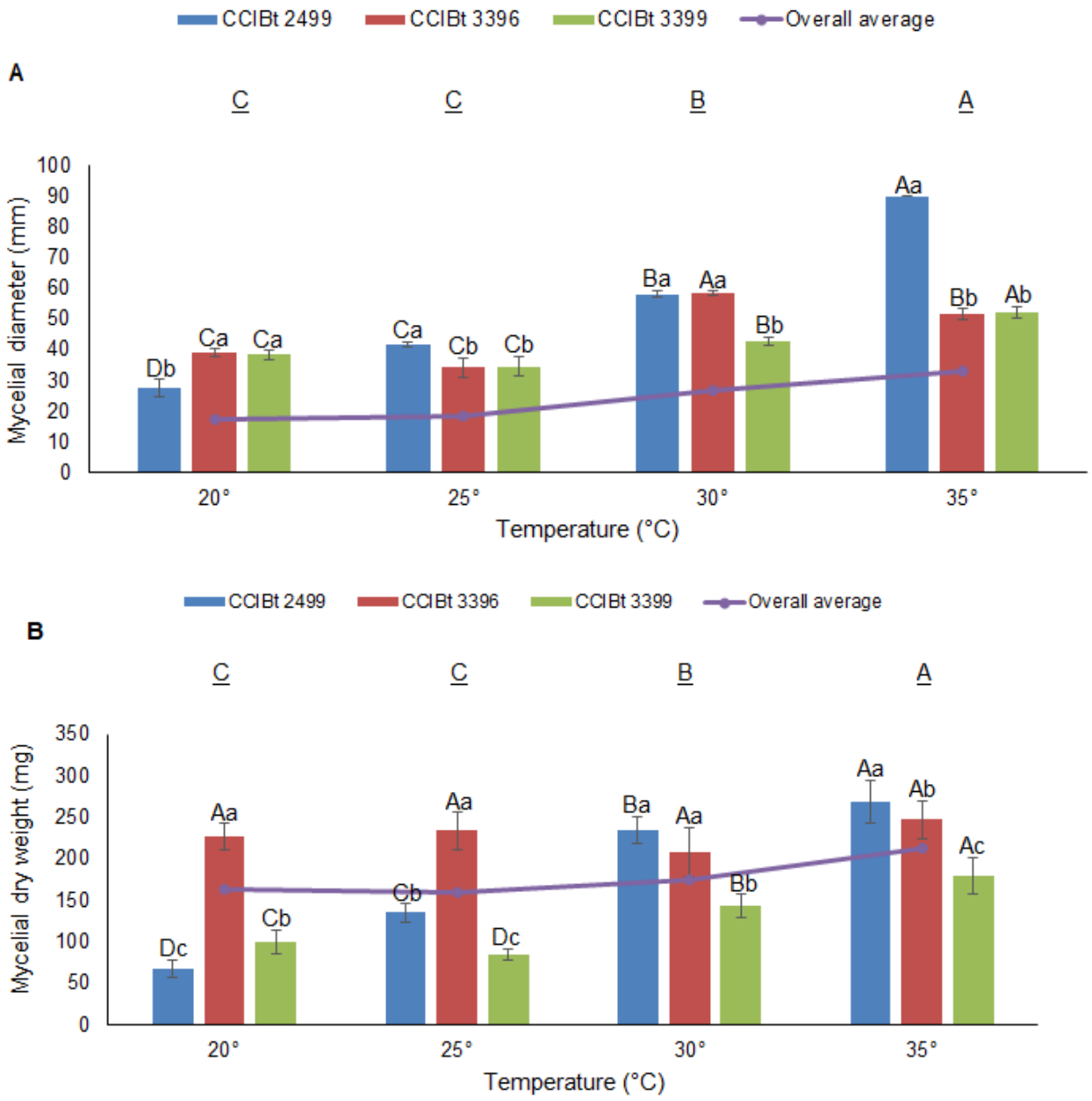

Figure 6. Mycelial growth of three wild isolates of *Panus strigellus* on the fourth day of incubation in PDA culture medium at different temperatures. A. Mycelial diameter (mm). B. Mycelial dry weight (mg). Lowercase letters compare different isolates at the same temperature. Capital letters compare the same isolate at different temperatures. Underlined capital letters compare the overall means of all isolates at different temperatures. Means followed by the same letter do not differ from each other using the Tukey test, at 5% probability.

Panus cf. *tephroleucus*

The isolate CCIBt3398 of *Panus* cf. *tephroleucus* showed highest (p≤0.05) mycelial growth at 30°C (Figure 7A), having completed the growth in PDA culture medium in five days, although the mycelial mass on that day was higher (p≤0.05) at 35°C, with 249.23 mg (± 18.52) of dry weight (Figure 7B).

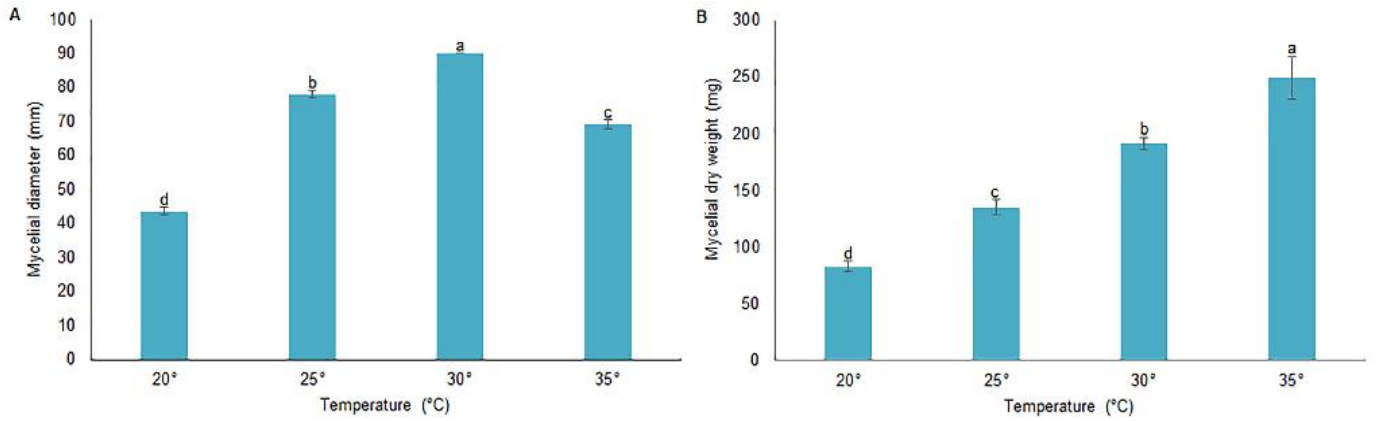

Figure 7. Mycelial growth of the wild isolate CCIBt3398 of *Panus* cf. *tephroleucus* on the fifth day of incubation in PDA culture medium at different temperatures. A. Mycelial diameter (mm). B. Mycelial dry weight (mg). Means followed by the same letter do not differ from each other using the Tukey test, at 5% probability.

Pleurotus albidus

The general average of mycelial growth of the six tested isolates of *Pl. albidus* showed statistically highest (p≤0.05) values at both 25°C and 30°C (Figure 8A), with no statistically difference (p>0.05) between these both temperatures. At 30°C, the isolate CCIBt2731 completed the Petri dish in six days and had the best (p≤0.05) mycelial growth (87.4 mm ± 2.25), which was higher (p≤0.05) than the growth of the same isolate at 25ºC (69.64 mm ± 1.82). At 25°C, the isolates CCIBt2405, CCIBt2731, and CCIBt4244 had statistically similar growth (p>0.05) among themselves, with lower (p≤0.05) values of growth at 30 ºC for the isolate CCIBt2405 and statically similar (p > 0.05) values at 30ºC for the isolate CCIBt4244. However, the highest (p≤0.05) dry weights of mycelial mass were presented by the isolates CCIBt2733 (134.41 mg ± 6.49) and CCIBt2844 (137.04 mg ± 9.07) at 30°C, with no statistical difference (p>0. 05) among them (Figure 8B).

Pleurotus djamor

The general average of mycelial growth (Figure 9A) of the seven tested isolates of *Pl. djamor* was statistically highest (p≤0.05) at both 25°C (67.9 mm ± 4.08) and 30°C (78.01 mm ± 4.36). The isolates CCIBt4719 and MPD704 had the best (p≤0.05) growth at 30°C, with the isolate CCIBt4719 having completed the plate in six days of growth. The values of mycelium growth of these both isolates did not have statistical difference (p>0.05) from the growth values at 25ºC. In relation to the mycelial mass on the sixth day of growth, the highest (p ≤ 0.05) average values of dry weight of all isolates were observed at 30°C, highlighting the isolate CCIBt4719 that have statistically highest biomass production at this temperature.

Pleurotus pulmonarius

The isolate CCIBt2963 of *Pl. pulmonarius* showed highest (p≤0.05) both mycelial growth (Figure 10A) and dry weight of mycelial mass (Figure 10B) at 30°C, having completed the plate in seven days.

DISCUSSION

For the genus *Lentinus*, all the tests showed temperatures higher than 25°C for the best mycelial development. For *L. concavus* and *L. berteroi*, this study is the first to investigate the mycelial growth and biomass production, and these results are important for further research on domestication and cultivation of these species. Marim and coauthors [33] and Colla and coauthors [34] tested isolates of *L. crinitus* from the state of Paraná, Southern Brazil, and both researches had good results at temperatures between 34°C and 37°C. In the present study, the best results were verified for the isolate CCIBt4790 of *L. crinitus* that showed the best mycelial growth and biomass production at 40°C.

The effect of temperature for the mycelial growth of genus *Panus* from Brazil has been studied by Vargas-Isla and Ishikawa [8], who evaluated an isolate of *P. strigellus* (named as *L. strigosus* Fr. but after reidentified as *P. strigellus* by Vargas-Isla and coauthors [35]) from the state of Amazonas, Northern Brazil. In that study, the authors evidenced 35°C as the best temperature for mycelial growth in PDA, which corroborates the results of the present study with emphasis for an isolate from Southeastern Brazil tested here. For *Panus* cf. *tephroleucus*, this is the first work to investigate the mycelial growth and biomass production, highlighting 30ºC as the best temperature for mycelial growth and 35ºC for biomass production based on an isolate from

the state of Amazonas, Northern Brazil. The sequence was initially blasted and had a greater affinity with *Panus velutinus*, but more recent molecular studies have revealed that the specimen may be close to *Panus tephroleucus* [27], although a careful taxonomic and morphological investigation is necessary to confirm the identity of this taxa.

For the genus *Pleurotus*, Lechner and Albertó [36] conducted tests of mycelial growth with Argentinean isolates of three species: *Pl. albidus*, *Pl. djamor*, and *Pl. pulmonarius*. In that article, the authors tested the mycelial growth of the isolates in Agar Noble culture medium at 5ºC, 12ºC, 20ºC, 25ºC, 30ºC, and 35ºC and evidenced that most of the isolates of the three species grew better at 25°C, with the exception of a *Pl. albidus* isolate and a *Pl. pulmonarius* isolate that grew better at 30ºC. In the tests carried out in this work for *Pl. albidus*, of the six isolates tested, the isolate CCIBt2731 from the Atlantic Forest in Southeastern Brazil had the highest mycelial growth at both 25ºC and 30ºC, whilst the isolates CCIBt2733 and CCIBt2844, both from Southeastern Brazil, had a better biomass production at 30ºC. For *Pl. djamor*, the average mycelial growth of the seven isolates tested in this work was better at both 25ºC and 30ºC, with emphasis on the isolate CCIBt4719 from the Pantanal biome, Midwest Brazil, that produced more biomass at 30ºC. For the isolate of *Pl. pulmonarius* from the Atlantic Forest in Southeastern Brazil studied in this work, the best both mycelial growth and biomass production were observed at 30°C.

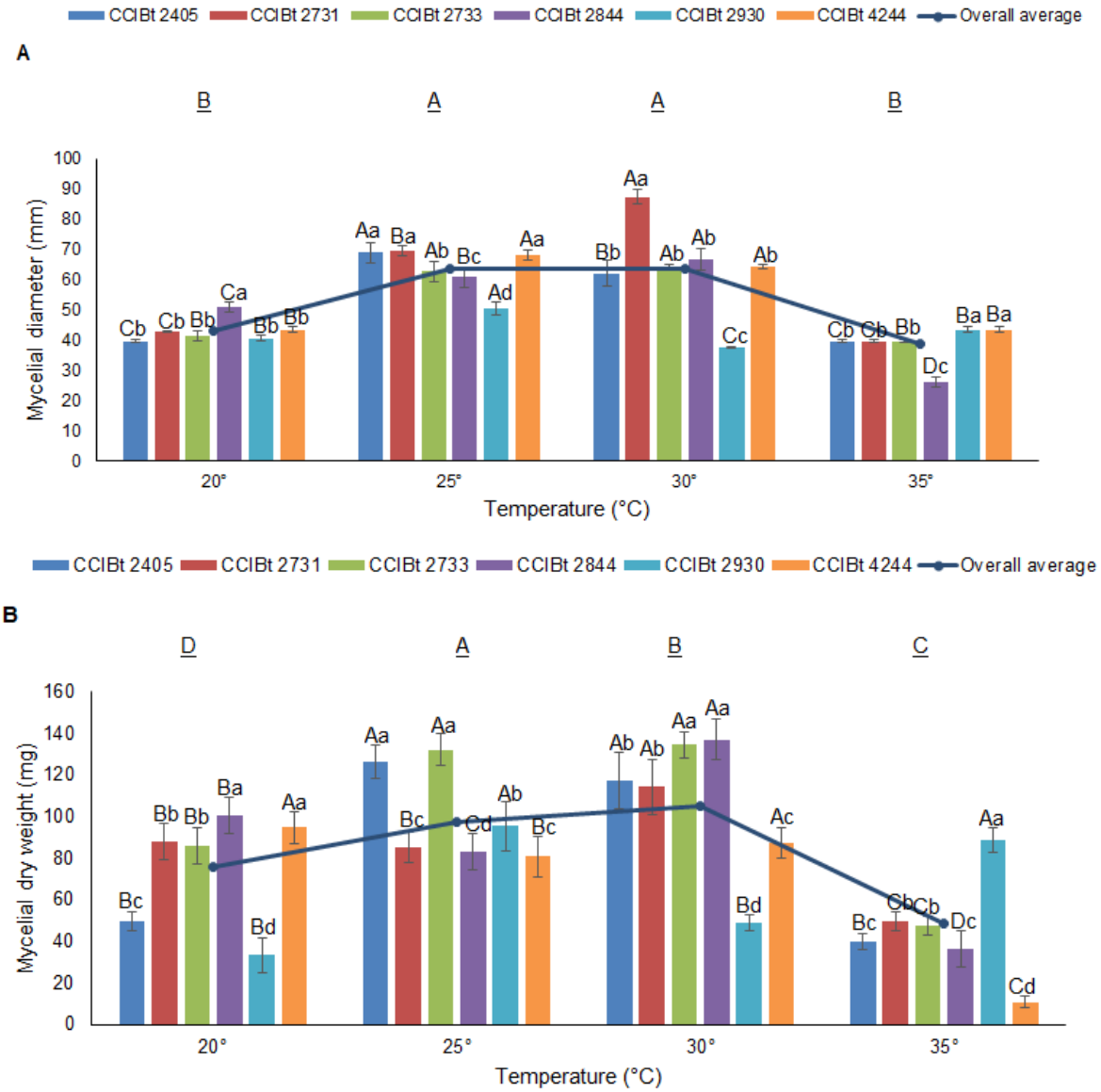

Figure 8. Mycelial growth of six wild isolates of *Pleurotus albidus* on the sixth day of incubation in PDA culture medium at different temperatures. A. Mycelial diameter (mm). B. Mycelial dry weight (mg). Lowercase letters compare different isolates at the same temperature. Capital letters compare the same isolate at different temperatures. Underlined capital letters compare the overall means of all isolates at different temperatures. Means followed by the same letter do not differ from each other using the Tukey test, at 5% probability.

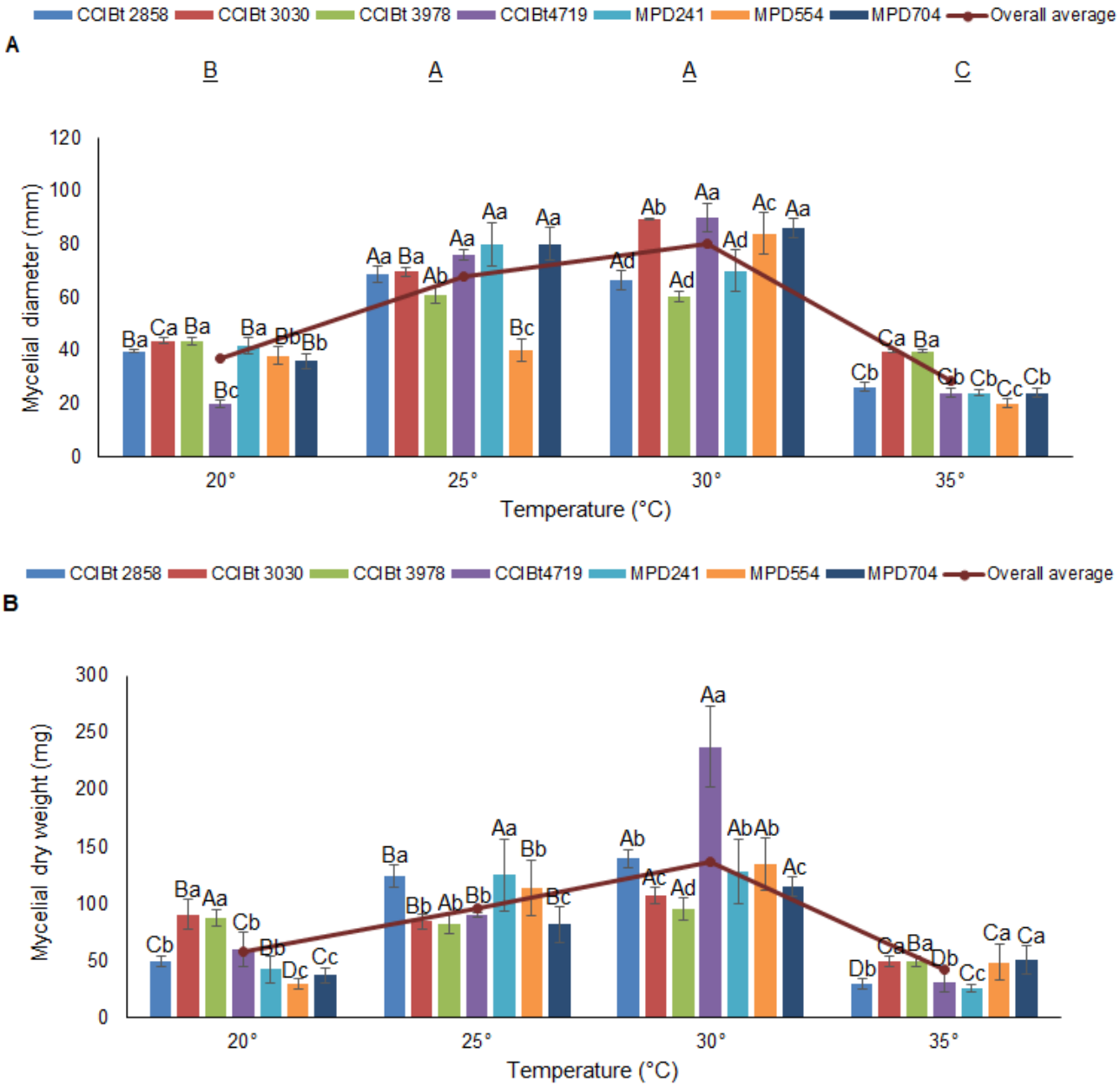

Figure 9. Mycelial growth of seven wild isolates of *Pleurotus djamor* on the sixth day of incubation in PDA culture medium at different temperatures. A. Mycelial diameter (mm). B. Mycelial dry weight (mg). Lowercase letters compare different isolates at the same temperature. Capital letters compare the same isolate at different temperatures. Underlined capital letters compare the overall means of all isolates at different temperatures. Means followed by the same letter do not differ from each other using the Tukey test, at 5% probability.

Mycelial growth tests are important to understand the colonization behavior of species and individuals at different temperature ranges and to begin one of the domestication steps for the cultivation of wild edible mushrooms. In this sense and as previously indicated, Mswaka and Magan [37] considered three groups of fungi based on the optimal growth temperature range: the low temperature group (25-30ºC), the intermediate group (30-37ºC), and the high temperature group (37-40ºC up to 55ºC). In this work, were considered the temperature range from 25°C to 35 °C as the best for the eight lentinoid and pleurotoid species studied, and following the classification of Mswaka and Magan [37], were included the studied *Pleurotus* species in the low temperature group, with best growth in the range from 25°C to 30°C, and the species of *Lentinus* and *Panus* in the intermediate group, with best growth from 30°C to 35°C.

These results also bring the possibility of reducing production costs in tropical climate areas such as Brazil when compared to other commercial mushroom species because most of the species herein tested grew at temperatures above 30°C, and can be produced in rustic greenhouses, without the need for climate adaptations that would make production more expensive.

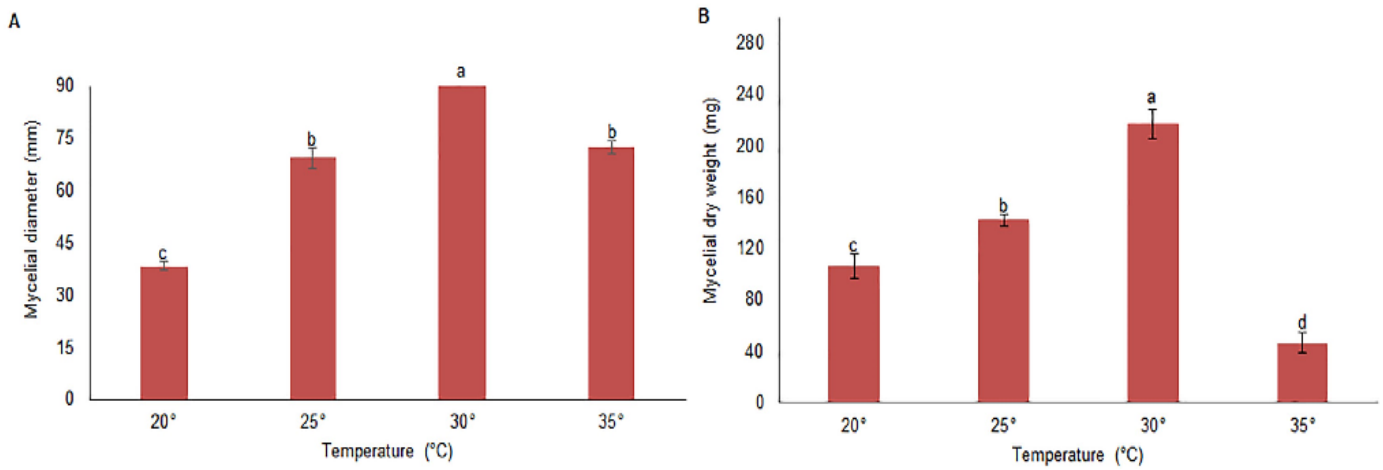

Figure 10. Mycelial growth of the wild isolate CCIBt2963 of *Pleurotus pulmonarius* on the seventh day of incubation in PDA culture medium at different temperatures. A. Mycelial diameter (mm). B. Mycelial dry weight (mg). Means followed by the same letter do not differ from each other using the Tukey test, at 5% probability.

CONCLUSION

This study aimed to determine the optimal temperature range for mycelial growth and biomass production of eight lentinoid and pleurotoid species of wild edible mushroom from Brazil. As a result, we highlight 25-30ºC for *Pl. albidus* and *Pl. djamor*, 30ºC for *Pl. pulmonarius*, 30-35ºC for *Panus* cf. *tephroleucus*, 35ºC for *L. berteroi*, *L. concavus*, and *P. strigellus*, and 35-40ºC for *L. crinitus* as the best temperature range for both mycelial growth and biomass production.

This study aims to pave the way for further research into the domestication and cultivation of wild edible mushroom species. Based on that, we intend to discover isolates of wild mushrooms that can be cultivated and are better adapted to the tropical climate conditions that are found in Brazil to a future commercial introduction of new mushroom species into the edible mushroom production chain.

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Conflicts of Interest: The authors declare no conflicts of interest. All the experiments undertaken in this study comply with the current laws of the country where they were performed.

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