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Cultural and ligninolytic activity studies of some polypores (Basidiomycota) from brazilian Atlantic Forest, São Paulo State, Brazil

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ABSTRACT - (Cultural and ligninolytic activity studies of some polypores (Basidiomycota) from brazilian Atlantic Forest, São Paulo State, Brazil). During a fungi survey in the brazilian Atlantic Forest in São Paulo, São Paulo State, Brazil, polypores growing on decaying wood were collected and fragments of the basidiomata were used to obtain isolates in pure culture. A total of 37 isolates were obtained and 23 species were identified. Cultural features of *Bjerkandera atroalba*, *Henningsia brasiliensis*, *Rigidoporus undatus*, and *Steccherinum reniforme* were described for the first time. A total of 31 isolates were evaluated in terms of their ability to oxidize the Remazol brilliant blue R dye (RBBR) and guaiacol. The results of these tests indicate that all isolated species were associated with white rot in the wood. Laccase and peroxidase activities were detected by drop tests using solutions of α-naphthol and pyrogallol. Production of cellulases and siderophores was detected with carboxymethylcellulose (CMC) and chrome azurol S (CAS) agar, respectively.
Keywords: Agaricomycetes, laccase, Neotropical region, peroxidase, xylophilous fungi

RESUMO - (Estudos de culturas e atividade ligninolítica de alguns políporos (Basidiomycota) da Mata Atlântica brasileira, São Paulo, SP, Brasil). Durante um levantamento de fungos na Mata Atlântica brasileira do Estado de São Paulo, SP, Brasil, fungos poroides crescendo em madeira em decomposição foram coletados e fragmentos dos basidiomas foram utilizados para obter culturas puras. Um total de 37 isolados foram obtidos e identificados em 23 espécies. Características das culturas de *Bjerkandera atroalba*, *Henningsia brasiliensis*, *Rigidoporus undatus* e *Steccherinum reniforme* são descritas pela primeira vez. Um total de 31 isolados foram avaliados em termos da capacidade para oxidar os corantes Remazol brilliant blue R (RBBR) e guaiacol. Resultados destes testes indicaram que todas as espécies estudadas são associadas com produção de podridão branca na madeira. Atividades lacase e peroxidase foram detectadas pelo teste de gotas usando soluções de α-naphthol e pyrogallol. Produção de celulases e sideróforos foram detectadas com o ágar carboxymethylcellulose (CMC) e chrome azurol S (CAS), respectivamente.
Palavras-chave: Agaricomycetes, fungos xilófilos, lacase, peroxidase, Região Neotropical

Introduction

Morphology until the latter part of the last century was the main tool used to characterize and to develop the taxonomy and putative phylogeny of different fungi groups (Rajchenberg 2011). Agaricomycetes have been studied in culture for about 100 years, and published works such as Nobles (1965) made the first attempt to understand polypores on the basis of their cultural features studied in vitro. Her work stressed the importance of (i) ability to degrade wood (brown vs. white rot types) as a key element when considering generic delimitation and taxonomy, (ii) detailed macro- and microscopic features of cultures in vitro and (iii) mating system as a character of great importance that underlines the biology of each species. After this work, an increasing number of studies were developed recording those features for each taxon in the literature. Stalpers (1978) published a major work of cultural characteristics of Polyporales and Corticiales and Nakasone (1990) presented a cultural description of 277 wood-inhabiting basidiomycetes, Rajchenberg (2011) present a review of the available knowledge on nuclear behavior of the mycelium, mating system and number of nuclei of the basidiospores within polypore genera, among others.

For decades, the division based on the presence or absence of extracellular oxidase in cultures proposed

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by Nobles (1965) to differentiate the fungi with different capacities to degrade wood, white-rot fungi, which breakdown lignin and cellulose, and brown-rot fungi, which breakdown cellulose and hemicellulose, was recognized like a character that have diagnostic significance to separate different species of wood-inhabiting fungi (Lundell *et al.* 2010). A recent phylogenetic study by Riley *et al.* (2014) indicates that this simple dichotomy of white rot vs. brown rot does not properly reflect the diversity of mechanisms by which wood-rotting fungi obtain nutrition. This study suggest that a more nuanced categorization scheme is needed to describe wood decay by species that degrade all cell wall polymers.

Nevertheless, the study of features of fungal cultures isolated in axenic conditions and the qualitative detection of enzymes causing the decomposition of lignin is still valuable as an identification tool, and a first approach to describe the underlying biology and biochemistry of the fungi (Nakasone 1990). This approach is also very useful to select isolates with potential use in biotechnological, biotransformation and bioremediation processes (Machado *et al.* 2005).

In this way, this study aimed: (1) to expand the knowledge about the biology of polypores presents in the Parque Estadual da Cantareira and Parque Estadual da Serra do Mar, São Paulo State, Brazil, with culture studies, and (2) to increase the knowledge of the enzymatic activity of some brazilian fungi, which have been poorly studied to date.

Materials and methods

Study of pure cultures - Specimens were collected from August 2011 to February 2015 in the Parque Estadual da Cantareira (PEC), (23°32'36"S, 46º37'59"W) and Parque Estadual da Serra do Mar (PESM) - Núcleo Santa Virginia (23º24' to 23º17'S, 45º03' W), São Paulo, Brazil. The species identification was based on macroscopic and microscopic characters of basidiomata. The specimens were deposited in the herbarium SP. Cultures were obtained from fresh basidiomata and grown in Malt Extract Agar (MEA). The species codes describing the cultures follow the Species Code of Nobles (1965) with the modifications summarized by Nakasone (1990). The isolates were preserved in sterile distilled water (Castellani 1967, Burdsall & Dorworth 1994) and deposited in the Coleção de Culturas de Algas, Cianobactérias e Fungos (CCIBt) of the Instituto de Botânica, São Paulo, Brazil.

Enzymatic test - All the enzymatic tests were inoculated with a 5 mm diam. agar plug, always in triplicate. Plates were incubated at 25 °C in the dark for 14 days.

Remazol Brillant Blue - R (RBBR) decolorization test: was detected by formation of an orange zone in the culture medium containing 2% (w/v) MEA with 0.05 % RBBR (Sigma) (Machado *et al.* 2005).

Phenol-oxidases production (Laccase s.s.) - was Detected by formation of a reddish zone in the culture medium containing 0.2% sugarcane bagasse powder, 0.01% guaiacol (Sigma) and 1.6% agar (Nishida *et al.* 1998). The drop test (Stalpers 1978) was carried out on the same plate using: (a) α -naphthol solution to detect Laccase (0.1 M in 96% ethanol) and (b) pyrogallol solution to detect Lignin Peroxidase (equal parts of 4% hydrogen peroxide and 1% pyrogallol, freshly prepared). The production of Laccase and Lignin Peroxidase was detected by formation of a purple and a yellowish-brown zone in the culture medium, respectively.

Cellulase activity test - Was detected by formation of a whitish zone in the culture medium containing 25 ml of modified basal medium (per liter): 0.5 g ammonium tartrate, 0.5 g monobasic potassium phosphate (KH_2PO_4), 0.05 g magnesium sulfate ($MgSO_4$), 0.01 g calcium chloride dehydrate ($CaCl_2 2H_2O$), 0.01 g manganese sulfate ($MnSO_4$), 0.05 g cooper (II) sulfate pentahydrate ($CuSO_4 5H_2O$) (Kirk *et al.* 1978), 15 g agar, 0.01 g congo red and 1.0 g carboxymethylcellulose (CMC).

Detection of siderophores production by a modification of chrome azurol S (CAS) agar plate assay - CAS-blue agar was prepared follow the modification proposed by Milagres *et al.* (1999). The CAS reaction was detected by change the agar color from blue to orange.

Results and Discussion

Antrodiella semisupina (Berk. & M.A. Curtis) Ryvarden, Prelim. Polyp. Fl. East Africa: 261 (1980).

Description of culture in Stalpers (1978).

Species code: 2.3.8.24.25.32.36.39.43.44.53.54.55.60.

Comments: the velvety to woolly mat and unusually downy, white to yellowish-brown, the dimitic hyphal system with generative hyphae clamped and skeletal

hyphae in the aerial mat are features of this species studied in culture, which is consistent with the observations made by Stalpers (1978).

Cultures examined: BRASIL. São Paulo: SP, PEC, 28-II-2012, *V. Motato-Vásquez, M.C. Westphalen & A.C. Bolaños 225* (CCIBt 3871).

Bjerkandera atroalba (Rick.) Westphalen & Tomšovský, Mycological Progress14(100): 3 (2015) Figures 1-2, 9-14

Macromorphological characters - Plates covered in the 1st week. Advancing zone: regular, appressed, feathery, becoming finely cottony, white. Aerial mycelium: initially appressed, villose, flocculent, white to cream. Submerged mycelium: white to cream. Odor: undetermined, weak to absent. Reverse: whitening.

Micromorphological characters - Advancing zone: generative hyphae simple septate and clamped, hyaline, frequently branched, with smooth and thin to thick-walled, 2.0-5.0 µm diam. Aerial mycelium: formed by generative hyphae with clamps, smooth and thin-walled, very branched, 4.0-7.0 µm diam. Skeletal hyphae not observed. Chlamydospores: present in most colonies, intercalary and terminals, usually ellipsoid, rarely subglobose, with thin wall 12.5-20.0 × 9.0-14.0 µm diam.

Species code: 2.3.4.34.36.40.41.53.54.60.

Comments: Molecular analysis confirmed that *Tyromyces atroalbus* (Rick) Rajchenb. belongs to *Bjerkandera* P. Karst and is separated from *Tyromyces* P. Karst, which is supported by the morphological features, such as the darkening of the basidiomata when dried and distinctly monomitic hyphal system (Westphalen *et al.* 2015). This study presents the first description of the culture of *Bjerkandera atroalba* characterized by the formation of chlamydospores and a monomitic hyphal system with generative hyphae with clamps. This features are shared with the culture of *B. adusta* (Willd.) P. Karst. (as *Polyporus adustus* Willd.), another species of the genus, described by Nobles (1965). This results differ with descriptions of cultures of species of *Tyromyces* in which Stalpers (1978) described the presence of skeletal hyphae. This results presents additional data that supports the transfer of this species to *Bjerkandera*.

Cultures examined: BRASIL. São Paulo: SP, PEC, 27-VI-2012, *V. Motato-Vásquez, M.C. Westphalen & A.C. Bolaños 248* (CCIBt 3974).

Coriolopsis rigida (Berk & Mont.) Murrill, N. Amer. Fl. 9:75 (1908).

Description of culture in Neves (1998).

Species code: 2.3.4.8.32.36.37.39.41.42.54.55.

Comments: the fast growth, the downy to felty mat and the presence of exudates hyaline to yellowish in most colonies characterizes this species in culture. Although the hyphal system of the basidioma is trimitic, in culture it was only possible to find generative hyphae with clamps and brown skeletal hyphae. These results differ from those presented by Neves (1998), who described the presence of skeletal and connective hyphae in the culture.

Cultures examined: BRASIL. São Paulo: SP, PEC, 29-XI-2011, *V. Motato-Vásquez 42* (CCIBt 3876).

Datronia caperata (Berk.) Ryvarden, Mycotaxon 23: 172 (1985)

Description of culture in Bakshi *et al.* (1969).

Species code: 2.3.8.34.36.39.43.(44).54.60.

Comments: this species is characterized in culture by white mat and presence of terminal and intercalary chlamydospores.

Cultures examined: BRASIL. São Paulo: São Paulo, PEC, 29-XI-2011, *V. Motato-Vásquez 41* (CCIBt 3877).

Dichomitus setulosus (Henn.) Masuka & Ryvarden, Mycol. Res. 103(9): 1130 (1999)

Description of culture in Rajchenberg (1983) as *Megasporoporia setulosa* (Henn.) Rajchenb.

Species code: 2.3.4.8.32.36.38.40.43.44.50.54.60.

Comments: this culture presents a dextrinoid reaction in the skeletal hyphae, which is a feature that generally have been used to identify species of this genus (Ryvarden 2004). The same reaction was observed by Neves (1998) in cultures of *D. cavernulosus* (Berk.) Masuka and Ryvarden, both species morphologically related. Dextrinoid reaction is absent in cultures of *D. hexagonoides* (Speg.) Robledo & Rajchenb. like observed by Stalpers (1978), and Rajchenberg (1983) also noted that *D. setulosus* and *D. hexagonoides* differ in the mat texture and the presence of distinct submerged generative hyphae, characters that could be useful to distinguish between both species. However, other biological and phylogenetic studies are needed to assist in the delimitation of species of the genus *Dichomitus* D.A. Reid.

Cultures examined: BRASIL. São Paulo: SP, PEC, 7-III-2012, *V. Motato-Vásquez 151* (CCIBt 3872).

Fomes fasciatus (Sw.) Cooke, Grevillea 14(69): 21 (1885)

Description of culture in Neves (1998).

Species code: 2.3.7.(8).(11).21.22.24.25.34.35.39.42.45.51.53

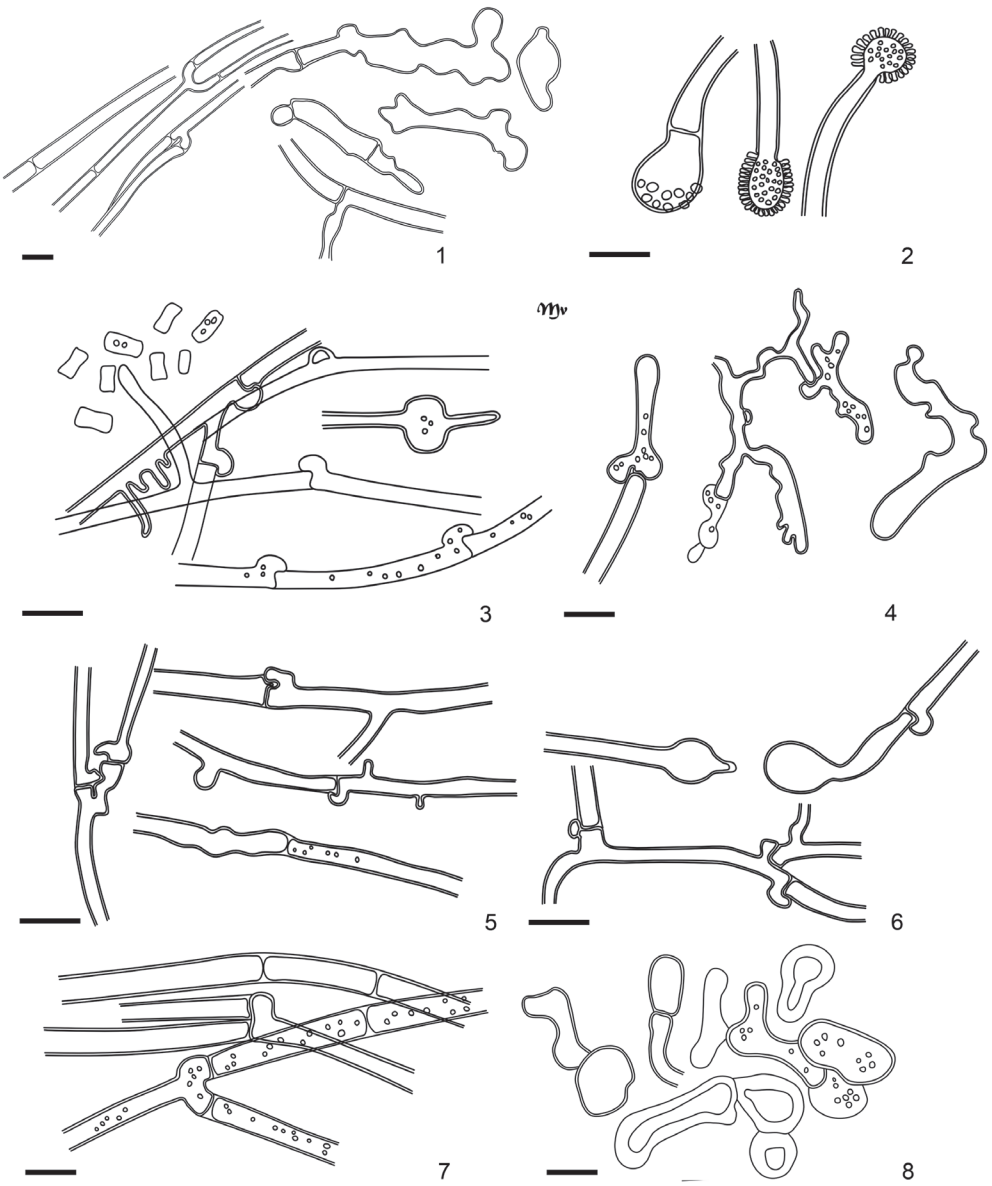
Comments: the culture studied is characterized by white to yellowish orange mat, becoming ochraceous. Broadly, our results agree with those obtained by Stalpers (1978) and Neves (1998). However, Neves (1998) noted in the cultures that the generative hyphae are lengthened in the colony margin to form a globular pseudoparenchyma, feature not observed in our culture.

Cultures examined: BRASIL. São Paulo: SP, PESM, 18-IV-2013, *R.M. Pires 34* (CCIBt 4082).

Fomitella supina (Sw.:Fr.) Murr. Bull. Torrey Bot. Club 32: 365 (1905)

Description of culture in Neves (1998).

Species code: 2.3.8.32.36.38.50.54.



Figures 1-8. Microscopic features. 1-2. *Bjerkandera atroalba*. 1. Generative hyphae. 2. Chlamydospores. 3-4. *Henningsia brasiliensis*. 3. Generative hyphae. 4. Cuticular cells. 5-6. *Rigidoporus undatus*. 5. Generative hyphae. 6. Cystidia. 7-8. *Steccherinum reniforme*. 7. Generative hyphae and arthroconidia. 8. Cuticular cells. Scale bar = 5 μm.

Comments: *Fomitella supina* is morphologically similar in culture with species of *Fomitopsis* P. Karst., except by the production of white rot in the former and the production of chlamydospores in the latter (Stalpers 1978, Neves 1998).

Cultures examined: BRASIL. São Paulo: SP, PESM, 17-IV-2013, *R.M. Pires 28* (CCIBt 4081).

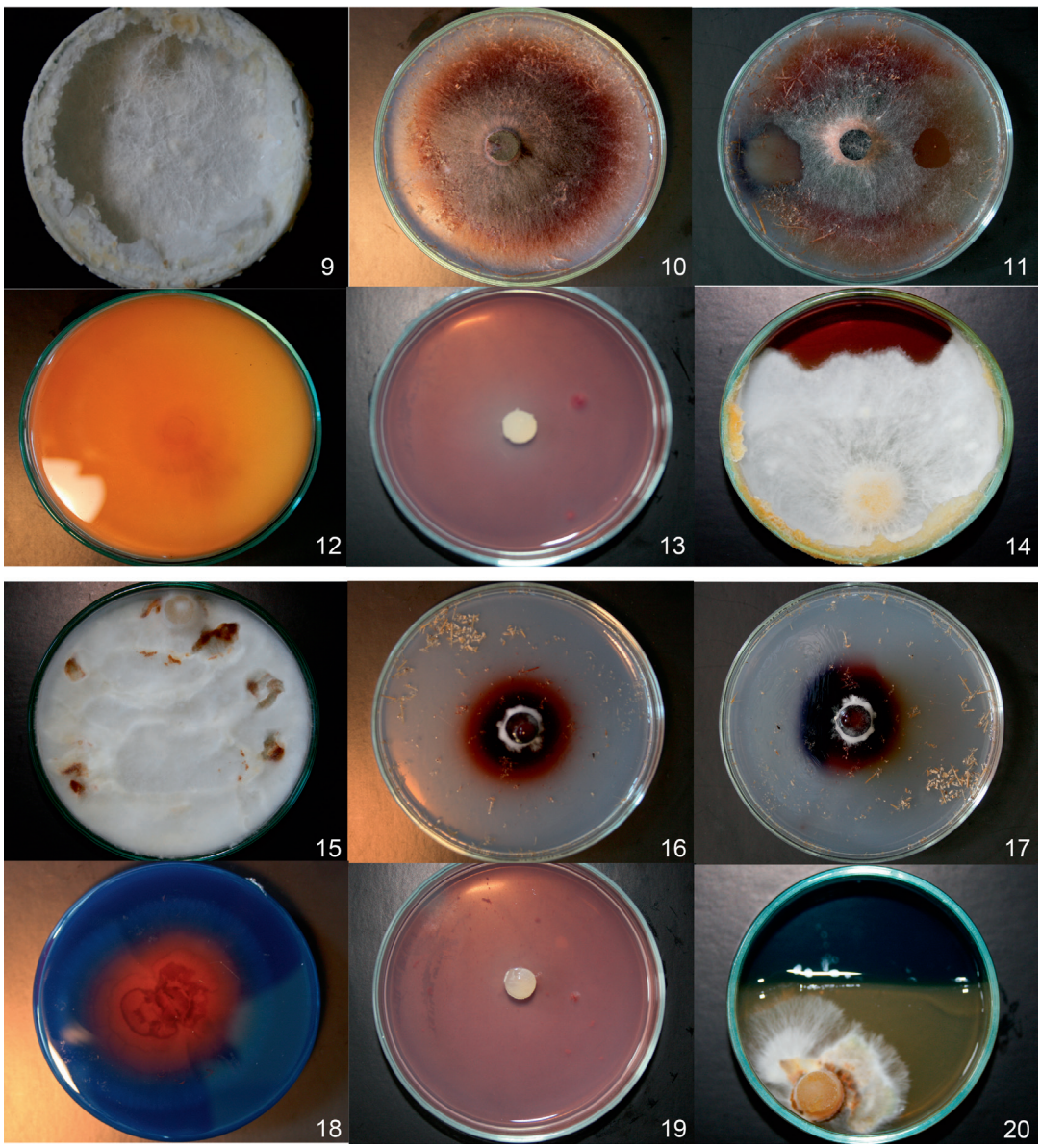
Fomitiporia maxonii Murrill, N. Amer. Fl. 9(1): 11 (1907).

Description of culture in Nobles (1965).

Species code: 2.6.8.11.34.37.39.45.46.50.54.57.

Comments: the culture of this species is characterized in culture by white and then dark brown mat with small floccose areas. Microscopically the presence of hyaline to yellowish generative hyphae, simple septate, hyaline to brown skeletal hyphae, globose to ovoid chlamydospores and irregular cells forming a pseudoparenchyma are characteristic.

Cultures examined: BRASIL. São Paulo: SP, PEC, 6-XII-2011, *V. Motato-Vásquez 76* (CCIBt 3874).



Figures 9-20. Enzymatic tests. 9-14. *Bjerkandera atroalba*. 9. Mycelial growths during six weeks. 10. Guaiacol oxidation. 11. Drop test. 12. RBBR decolorization. 13. Cellulase production, non-detected. 14. Siderophores production. 15-20. *Henningsia brasiliensis*. 15. Mycelial growths during six weeks. 16. Guaiacol oxidation. 17. Drop test. 18. RBBR decolorization. 19. Cellulase production, non-detected. 20. Siderophores production, non-detected. Plate = 9 cm.

Ganoderma australe (Fr.) Pat., Bull. Soc. Mycol. France 5: 71 (1889).

Description of culture in Bettucci & Guerrero (1971).

Species code: 2.3.8.10.32.37.39.(40).46.47.50.54.55.

Comments: this species is very variable in culture, feature observed in the three different isolates obtained from different basidiomata. But it usually can be recognized by the slow growth, cracking the agar medium and wrinkling the colony surface. Besides of the mat white and the abundant sphaerical cuticular cells, features that were also observed by Bettucci & Guerrero (1971) and Yeh *et al.* (2000).

Cultures examined: BRASIL. São Paulo: SP, PEC, 6-XII-2011, *V. Motato-Vásquez 72* (CCIBt 3972), 28-II-2012, *V. Motato-Vásquez 124* (CCIBt 3973); PESM, 11-VI-2013, *R.M. Pires 57* (CCIBt 4086).

Henningsia brasiliensis (Speg.) Speg., Bol. Acad. Nal. Cienc. Córdoba 23(3-4): 411 (1919).
Figures 3-4, 15-20

Macromorphological characters - Plates covered in 5-6th week. Advancing zone: smooth, appressed, subfelty, white. Margin uneven. Aerial mycelium: cottony to woolly and later downy, initially white and later forming an orange to reddish-orange crust. Submerged mycelium: white to cream. Odor: undetermined. Reverse: unchanged.

Micromorphological characters - Advancing zone: generative hyphae simple septate, hyaline, straight, branched, with smooth and thin-walled, 1.5-3.0 µm diam. Aerial mycelium: as in the advancing zone. Pseudoparenchyma: hyphae differentiated, with thickened walls presents in the crusty areas. Submerged mycelium: as in the advancing zone.

Species code: 2.6.10.32.36.(37).38.45.46.53.54.

Comments: *Henningsia brasiliensis* has been closely associated with the genus *Rigidoporus* Murrill with regard to their morphological characteristics. In culture, it is possible to relate the two genera by the coloration of the mat and the formation of pseudoparenchyma. But in the culture, *H. brasiliensis* could be separated microscopically by the absence of cystidia, when compared with the two species of *Rigidoporus* studied here: *R. lineatus* (Pers.) Ryv. and *R. microporus* (Fr.) Overeem).

Cultures examined: BRASIL. São Paulo: SP, PEC, 7-XI-2012, *V. Motato-Vásquez et al. 280* (CCIBt 3976).

Inonotus tropicalis (M.J. Larsen & Lombard) T. Wagner & M. Fisch., Mycologia 94(6): 1009 (2002)

Description of culture in Larsen & Lombard (1988)

Species code: 2.6.7.(17).32.37.39.43.54.

Comments: the moderately rapid growth on MEA, the yellow mat, often pale brown generative hyphae, simple-septate, dark brown skeletal hyphae, rare or absent, brown and narrowly subulate setae are characteristics of this species in culture, which are consistent with the observations carried out by Larsen & Lombard (1988).

Cultures examined: BRASIL. São Paulo: SP, PESM, 12-VI-2013, *R.M. Pires 73* (CCIBt 4130).

Junghuhnia nitida (Pers.) Ryvarden, Persoonia 7(1): 18 (1972)

Description of culture in Stalpers (1978).

Species code: 2.3.8.9.(11).13.(14).24.25.32.36.(38).39.42.52.54.57.

Comments: the cultural features of our specimen are quite similar to those found by Stalpers (1978). The white mat with salmon pores after 3-5 weeks, encrusted skeletal hyphae and variably present cystidia sometimes present, apically somewhat swollen are characteristics of this species in culture.

Cultures examined: BRASIL. São Paulo: SP, PESM, 12-VI-2013, *R.M. Pires 79* (CCIBt 4092).

Oxyporus latemarginatus (Durieu & Mont.) Donk, Persoonia 4(3): 342 (1966)

Description of culture in Lombard *et al.* (1960).

Species code: 2.4.7.10.35.36.38.41.50.54.55.

Comments: the rapid growth on MEA, absence of clamps, generative hyphae often encrusted and absence of cystidia are characteristics of this species in culture. The results presented here agree with those reported by Lombard *et al.* (1960).

Cultures examined: BRASIL. São Paulo: SP, PEC, 7-XI-2012, *V. Motato-Vásquez et al. 283* (CCIBt 3975).

Polyporus tenuiculus (P. Beauv.) Fr., Syst. Mycol. 1: 344 (1821)

Description of culture in Neves (1998).

Species code: 2.3.8.35.36.38.45.46.53.54.60.

Comments: the cottony and white mat, the dimitic hyphal system with clamped generative hyphae, branched skeletal hyphae and abundant arthroconidia characterizes the culture of this species. Neves (1998) observed the presence of crystals in the cultures studied, this results differ from our observations and largely with those made by Stalpers (1978).

Cultures examined: BRASIL. São Paulo: SP, PEC, 24-VIII-2012, *V. Motato-Vásquez & M.C. Westphalen 275* (CCIBt 3963).

Pycnoporus sanguineus (L.) Murrill, Bull. Torrey Bot. Club 31(8): 421 (1904)

Description of culture in Nobles (1965).

Species code: 2.3.8.34.35.36.38.41.42.53.54.55.60.

Comments: the strong orange to pinkish-salmon color of the culture is similar to the color of the basidioma. This is a very important characteristic for easy recognition of the species in culture. Our results agree with those presented by Nobles (1965), Bettucci & Guerrero (1971) and Wright & Deschamps (1975). Neves (1998) observed the formation of chlamydospores and rhomboid crystals near the mat surface, characteristics absent in the cultures studied in this work.

Cultures examined: BRASIL. São Paulo: SP, PEC, 30-VII-2012, *V. Motato-Vásquez & M.C. Westphalen 260* (CCIBt 3962); PESM, 7-VIII-2013, *R.M. Pires 71* (CCIBt 4090).

Rigidoporus lineatus (Pers.) Ryvarden, Norw. J. Bot. 19: 236 (1972)

Description of culture in Wright & Deschamps (1975).

Species code: 2.6.10.13.32.36.39.43.44.51.54.55.

Comments: the initially white and then orange mat, simple septate generative hyphae, pseudoparenchyma formed by swollen generative hyphae, the spiny or smooth and clavate cystidia, (13.5-18.5 × 6.0-14.5 µm) are characteristics of this species.

Cultures examined: BRASIL. São Paulo: SP, PEC, 29-XI-2011, *V. Motato-Vásquez 47* (CCIBt 3959).

Rigidoporus undatus (Pers.) Donk, Persoonia 5(1): 115 (1967).
Figures 5-6, 21-26

Macromorphological characters: plates covered in 5-6th week. Advancing zone: regular, smooth, and white. Margin uneven. Aerial mycelium: initially white, later pinkish-salmon, appressed, and velvety to downy. Submerged mycelium: white to cream. Odor: acid. Reverse: yellowish-brown.

Micromorphological characters: Advancing zone: generative hyphae simple septate, hyaline, straight, branched, with smooth and thin wall, 2.0-4.5 µm diam. Aerial mycelium: as the advancing zone. Pseudoparenchyma: differentiated hyphae with thickened walls present in the crusty areas. Cystidia: clavate, spiny or smooth, 15.5−22.5 × 7.0−17.5 µm. Submerged mycelium: as the advancing zone. Odor: like iodoform. Reverse: unchanged.

Species code: 2.6.10.13.32.36.39.46.47.52.54.55.

Comments: comparing the culture of *R. undatus* with another cultures of species of the genus, they share the presence of simple septate generative hyphae, single cells differentiated in the pseudoparenchyma and presence of cystidia. The differentiation between the species of *Rigidoporus* in culture is difficult, but in this study we can separate *R. lineatus* from *R. undatus* based on growth rate (2-4 weeks in the former and 6-7 weeks in the latter) and the size of cystidia (13.5-18.5 × 6.0-14.5 µm in the former and 15.5-22.5 × 7.0-17.5 µm in the latter).

Cultures examined: BRASIL. São Paulo: SP, PEC, 29-XI-2011, *V. Motato-Vásquez 38* (CCIBt 3958).

Schizopora flavipora (Berk. & M.A. Curtis *ex* Cooke) Ryvarden, Mycotaxon 23: 186 (1985)

Description of culture in Stalpers (1978).

Species code: 1.3.32.36.40.43.44.53.54.55.60.

Comments: this species is characterized in culture by white and thin mat, initially farinaceous to villose and microscopically by the generative hypha with abundant clamps.

Cultures examined: BRASIL. São Paulo: SP, PEC, 27-VII-2012, *V. Motato-Vásquez, M.C. Westphalen & A.C. Bolaños 256* (CCIBt 3965).

Steccherinum reniforme (Berk. & M.A. Curtis) Banker, Mem. Torrey Bot. Club 12: 127 (1906)
Figures 7-8, 27-32

Macromorphological characters: plates covered in the 1st week. Advancing zone: smooth, appressed, cottony, white. Margin: uneven. Aerial mycelium: appressed, cottony to downy, white to yellowish. Submerged mycelium: white to cream. Odor: undetermined. Reverse: bleached.

Micromorphological characters: Advancing zone: generative hyphae with clamps, hyaline, straight, smooth, thin-walled, 1.5-2.5 µm diam. Aerial mycelium: as in the advancing zone. Arthroconidia: hyaline, smooth, thick-walled. Submerged mycelium: as in the advancing zone and hyphae differentiated through, hooked, thick-walled, and interlocked to form plectenchyma.

Species code: 2.3.11.35.36.40.41.53.54.

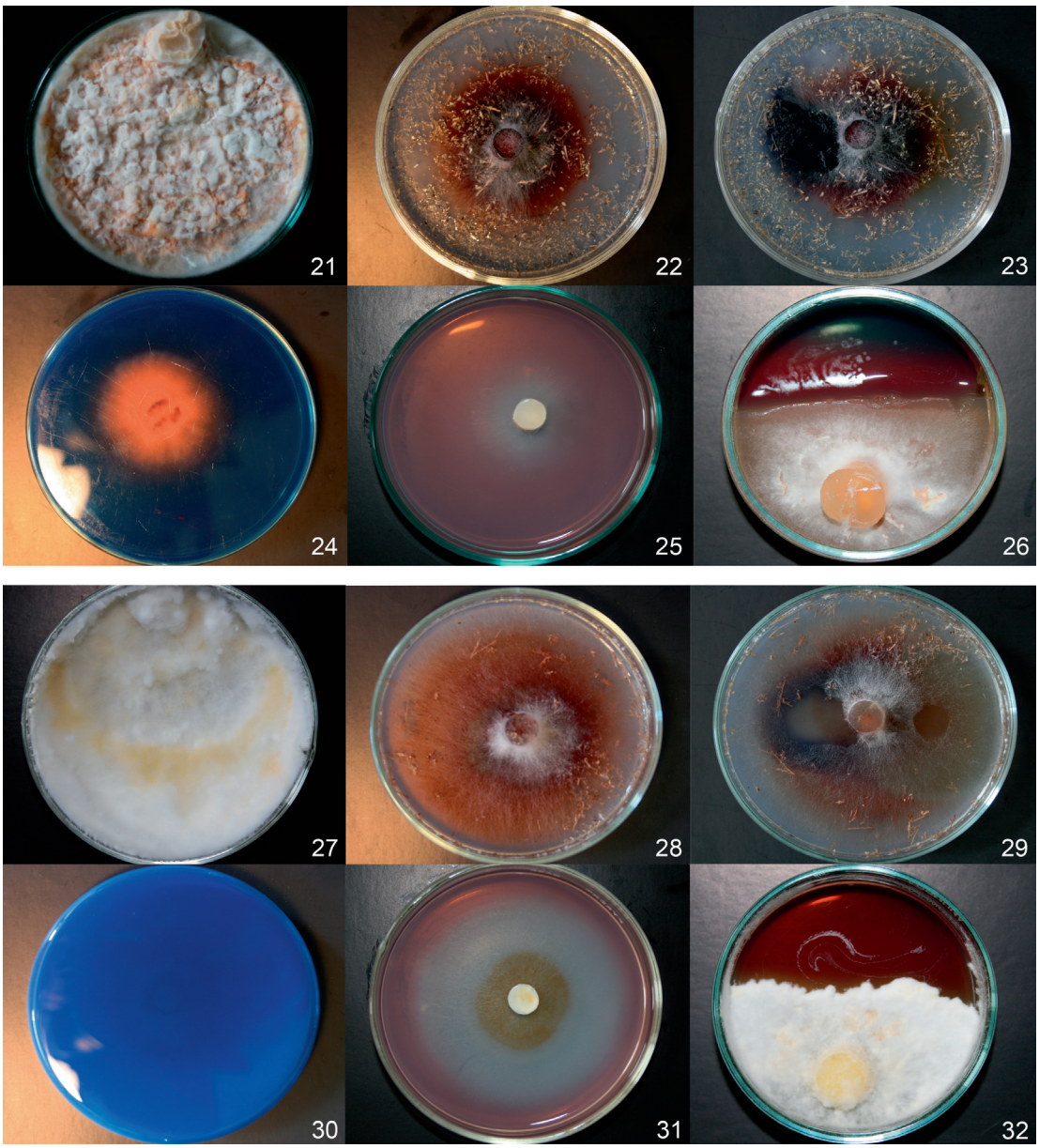
Comments: colonies growing very fast, mat white to yellowish, cottony, clamped generative hyphae, arthroconidia hyaline, smooth and with thick wall are characteristics of this species.

Cultures examined: BRASIL. São Paulo: SP, PEC, 30-VIII-2012, *V. Motato-Vásquez & M.C. Westphalen 263* (CCIBt 3971).

Tinctoporellus empimiltinus (Berk. & Broome) Ryvarden, Trans. Br. Mycol. Soc. 73: 19 (1979)

Description of culture in Rajchenberg (1983).

Species code: 2.4.8.10.11.34.35.37.38.(39).41.50.54.



Figures 21-32. Enzymatic tests. 21-26. *Rigidoporus undatus*. 21. Mycelial growths during six weeks. 22. Guaiacol oxidation. 23. Drop test. 24. RBBR decolorization. 25. Cellulase production. 26. Siderophores production. 27-32. *Steccherinum reniforme*. 27. Mycelial growths during six weeks. 28. Guaiacol oxidation. 29. Drop test. 30. RBBR decolorization, non-detected. 31. Cellulase production. 32. Siderophores production. Plate = 9 cm.

Comments: *Tinctoporellus epimiltinus* showed similar growth and microscopic characteristics of *Grammothele subargentea* (Speg.) Rajchenb. in culture medium (Rajchenberg 1983). Both change the color of the culture medium, making it reddish and form a plectenchyma. The basidioma of both species exhibit a similar hyphal system, with dextrinoid skeletal hyphae and the production of white rot in the substrate. In addition, different species of the genus *Grammothele* Berk. & M.A. Curtis have also been transferred or synonymized with *Porogramme* (Pat.) Pat. species (see Soares *et al.* 2015), which also indicates the narrow morphological and ecological relationship of these three genus and the need for taxonomic and phylogenetic revision with more specialized techniques like cytological and sexual studies as commented by Rajchenberg (1983).

Cultures examined: BRASIL. São Paulo: SP, PEC, 29-XI-2011, *V. Motato-Vásquez 46* (CCIBt 3875), 28-II-2012, *V. Motato-Vásquez 122* (CCIBt 3873).

Trametes elegans (Spreng.) Fr., Epicr. Syst. Mycol: 491 (1838)

Description of culture in Nobles (1965).

Species code: 2.3.4.8.34.35.36.40.41.(42).50.54.

Comments: the culture of this species was examined and it was found to differ from the cultures of another species of *Trametes* Fr. that were examined by Stalper (1978) and Neves (1998) and also in this work, principally by the presence of chlamydospores and arthroconidia besides of the generative hyphae simple-septate in the advance zone.

Cultures examined: BRASIL. São Paulo: SP, PEC, 6-XII-2011, *V. Motato-Vásquez 67* (CCIBt 3960), 28-II-2012, *V. Motato-Vásquez 131* (CCIBt 3961), 27-VI-2012, *V. Motato-Vásquez, M.C. Westphalen & A.C. Bolaños 243* (CCIBt 3964).

Trametes villosa (Sw.) Kreisel, Monogr. Cienc. Univ. Habana 16: 83 (1971)

Description of culture in Susin (1989).

Species code: 2.3.8.32.36.40.41.42.50.54.55.60.

Comments: the results obtained in this study are similar to those obtained by Susin (1989) and Neves (1998), except that our culture not present connective hyphae and the reverse was not bleaching. The generative hyphae in the margin presents clamps and chlamydospores was absent, different to the culture of *T. elegans*.

Cultures examined: BRASIL. São Paulo: SP, PEC, 20-IX-2011, *V. Motato-Vásquez & A.M. Gugliotta 8, 9* (CCIBt 3966, CCIBt 3967), 29-XI-2011, *V. Motato-Vásquez 48* (CCIBt 3968), 8-V-2012, *V. Motato-Vásquez 204* (CCIBt 3969); PESM, 11-VI-2013, *R.M. Pires 55* (CCIBt 4084), 12-VI-2013, *R.M. Pires 74* (CCIBt 4091), 13-VI-2013, *R.M. Pires 88* (CCIBt 4093), 8-VIII-2013, *R.M. Pires 118* (CCIBt 4133).

Trichaptum sector (Ehrenb.) Kreisel, Monogr. Cienc. Univ. Habana 16: 84 (1971)

Description of culture in Alexander *et al.* (1989).

Species code: 2.3.7.32.36.38.43.54.60.61.

Comments: colonies growing moderately fast, white and subfelty mat, clamped generative hyphae, and intercalary or terminal arthroconidia are characteristics of this species.

Cultures examined: BRASIL. São Paulo: SP, PEMS, 11-VI-2013, *R.M. Pires 63, 67* (CCIBt 4088, CCIBt 4129).

Enzymatic test – A total of 37 specimens were isolated from basidiomata growing in decaying wood and classified into 23 species. Of all the isolated obtained, 31 were qualitatively evaluated in terms of their ligninolytic activity (table 1). All species were associated with production of a white rot with regard to the presence of Phenol-oxidases that was confirmed for almost all specimens tested. In this test only had negative results: *A. semisupina* (RBBR), *S. flavipora* (all tests), *I. tropicalis* (Guaiacol), *S. reniforme* (Laccase and RBBR) *T. sector* (Guaiacol) and *T. atroalbus* (laccase, peroxidase and RBBR). These species were also associate with production of a white rot from data obtained in the literature (Stalpers 1978, Larsen & Lombard 1988, Alexander *et al.* 1989). Currently it is known that negative results in this tests may be related to the lack of specificity of the enzymes in the oxidation of substrates tested or absence of expression of the total ligninolytic system due to the culture conditions used (Platt *et al.* 1985).

Production of cellulolytic enzymes was detected in 18 of the 31 isolates tested. In all the isolates the growth was very poor. These results agree with the literature, since the expression of the total cellulolytic activity could differ due to the culture conditions (Arantes & Milagres 2009).

Detection of siderophores in CAS-agar occurred only in 18 of the 31 isolates tested and they differed in terms of growth and change of CAS-agar color. It is believed that fungi that produced low molecular weight compounds are responsible for the initial process of degradation of wood (Arantes *et al.* 2011).

It should be noted that some of the isolates showing positive results in all the biochemical tests (*C. rigida*, *P. sanguineus*, *T. elegans* and *T. epimiltinus*) present records in the current literature being used in processes such as degradation of effluents and recalcitrant xenobiotic compounds (Gomes *et al.* 2009, Lyra *et al.* 2009). Other less commonly used species such as *Fomes fasciatus*, *F. supina*, *O. latemarginatus* and *R. undatus* also performed well and showed very fast growth, features that make them interesting for studies in biotechnological or bioremediation processes in laboratory scale. The results presented in this work suggested that these tests could be used as a simple and effective way to indirectly select fungi producers of lignocellulolytic enzymes, as an important basis for future biotechnological, ecological and molecular studies.

Table 1. Ligninolytic activity of polypores isolated from decaying wood. Production of enzymes: Phenol-Oxidase in Guaiacol (G) and RBBR test, production of Laccase (L), Peroxidase (P), Cellulase (C) and Siderophores (S). Detection of enzymes: positive (+) or negative (−).

Species	CCIBt	G	RBBR	L	P	C	S
A. semisupina	3871	+	−	+	+	+	−
B. atroalba	3974	+	+	−	−	−	+
C. rigida	3876	+	+	+	+	+	+
D. caperata	3877	+	+	+	+	−	−
D. setulosus	3872	+	+	+	+	+	−
F. fasciatus	4082	+	+	+	+	+	+
F. supina	4081	+	+	+	+	+	+
F. maxonii	3874	+	+	+	+	+	−
G. australe	3972	+	+	+	+	−	+
G. australe	3973	+	+	+	+	−	+
H. brasiliensis	3976	+	+	+	+	−	−
I. tropicalis	4130	−	+	+	+	+	−
J. nitida	4092	+	+	+	+	−	−
O. latemarginatus	3975	+	+	+	+	+	+
P. tenuiculus	3963	+	+	+	+	−	−
P. sanguineus	3962	+	+	+	+	+	+
R. lineatus	3959	+	+	+	+	+	−
R. undatus	3958	+	+	+	+	+	+
S. flavipora	3965	−	−	−	−	−	−
S. reniforme	3971	+	−	+	−	+	+
T. epimiltinus	3873	+	+	+	+	+	+
T. epimiltinus	3875	+	+	+	+	+	+
T. elegans	3960	+	+	+	+	+	+
T. elegans	3961	+	+	+	+	+	+
T. elegans	3964	+	+	+	+	−	+
T. villosa	3966	+	+	+	+	−	+
T. villosa	3967	+	+	+	+	−	+
T. villosa	3968	+	+	+	+	−	+
T. villosa	3969	+	+	+	+	−	+
T. sector	4088	−	+	+	+	+	–
T. sector	4092	−	+	+	+	+	–

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