



Identification of volatile organic compound producing Lignicolous fungal cultures from Gujarat, India

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ABSTRACT

This study aims to identify the lignicolous basidiomycetes species that synthesize volatile organic compounds with potential applications in food industry, cosmetics, perfumery and agriculture. We have collected fruiting bodies from different woody plants and the lignicolous basidiomycetes species were identified by their macroscopic and microscopic characteristics. From the context of the fresh fruiting bodies small fragments of dikaryotic mycelium were extracted and inoculated on PDA and MEA media for isolation and pure cultures are kept in dark at a temperature of 25°C. 11 species of lignicolous basidiomycetes, belonging to 6 families and 5 orders were isolated in pure culture. The isolates were analyzed in vitro and the main characteristics that were observed are: the general aspect of the surface and the reverse of the colonies, the changing in colour and the growth rate of the mycelium and also the specific odour which indicates the presence of the organic volatile compounds. For the first time lignicolous fungi like *Flavodon flavus* (Klotz.) Ryv., *Ganoderma lucidum* (Curtis) P. Karst, *Hexagonia apiaria* (Pers.) Fr., *Lenzites betulina* (L.) Fr. *Lenzites eximia* Berk. & M.A. Curtis, *Lenzites sterioides* (Fr.) Ryv., *Navisporus floccosus* (Bres.) Ryv., *Phellinus gilvus* (Schwein.) Pat., and *Trametes pini* (Thore) Britzelm. were producing characteristic smell that indicates presence of Volatile organic compounds. For the first time from Gujarat, India the Volatile organic compound producing fungi were identified and their culture characters also described.

Keywords: Volatile organic compound (VOC), Lignicolous fungi, *Lenzites sterioides*, India

1. INTRODUCTION

Lignicolous fungi represent a diversified group of fungi in terms of ecological and morphological aspect but it also represents a heterogeneous taxonomic group. The traditional methods of identifying the lignicolous fungi based on collecting and analyzing the fruit bodies present the disadvantage of not being able to identify the Basidiomycetes that do not form fruit bodies (due to local ecological and climatic conditions) or those with degraded fruit bodies and attacked wood often do not show fruiting bodies, so the identity of the responsible fungus was ascertained by pure cultures derived from wood.

The analysis of the macroscopical and microscopical features of the mycelium grown *in vitro* offer the possibility to identify species without using fruit bodies (when the fungi are isolated from substratum) and it also offers a valuable resources to taxonomic studies. Lignicolous Aphyllophorales in general do not form basidiocarps in pure culture. The taxonomy of this order is based on characters of the fruiting body. Identification of cultures with the conventional keys is almost impossible. But the most extensive existing key (53 characters,) of Nobles, (1965) new and more comprehensible code (96 characters) of Stalpers (1978) were used.

The cultural features of some isolated lignicolous fungi have been studied by Nobles, (1948); Stalpers (1978, 1993), Bakshi et al. (1969, 1970); Niemelä, (1975, 1977) have studied the culture characters of genera like *Fomes*, *Phellinus* and *Trametes*. In the present paper certain lignicolous fungal cultures have been identified and maintained as pure cultures.

Volatile organic compounds are synthesized by lignicolous fungi in pure cultures so the quantity and quality of the volatile organic compounds synthesized by lignicolous basidiomycetes depends on several abiotic factors such as the chemical composition and pH of the substrate (Chen et al., 1984; Ewen et al., 2004; Wheatley, 2002), temperature (Tronsmo & Dennis, 1978), water content, but also on some biotic ones, such as development stage (Fäldt et al., 1999; Wu et al., 2005) and the presence of other organisms (Hynes et al., 2007).

Taxonomically, many of these species are found in orders such as Agaricales, Boletales, Ganodermatales, Hymenochaetales, Polyporales and Stearales, many of them being lignicolous macromycetes (Suay et al., 2000). Several studies have shown the great potential of lignicolous basidiomycetes in bio-prospecting. In the last few years, the scientific interest towards volatile organic compounds has grown not only for their ecological role in interspecific and intraspecific communication, defense, but also for their great bioactive potential in various industries, ranging from food industry, cosmetics and perfumery, pharmaceutical industry and agriculture, in the biocontrol of plant pathogens as biopesticides (Morath et al., 2012).

The main objective of this study is to identify the species of lignicolous basidiomycetes that produce specific odours when grown on synthetic media, odours that indicate the presence of the volatile organic compounds.

2. MATERIALS AND METHODS

Study area

Gujarat is bounded by the Arabian Sea at the southwest and geographically located between 20° 6' N to 24° 42' N and 68° 10'E to 74° 28' E. The total geographical area of the state comprises of land mass of 1,96,204 km² (75,755 sq miles) from which a little or less than 20 lakh hector of land is under forest cover which is unevenly distributed but the major concentration is found on the eastern hilly Saurashtra region. The temperature of the state ranges from 1 °C to 46 °C. The high variation in geophysical and climatic conditions resulted in the formation of different forest types. The rainfall received in the state varies from region to region and the northern part of the state is a desert.

Isolation of Fungi

The fungi associated with the samples were isolated. Wood chips measuring 5 mm × 5 mm × 2 mm were aseptically removed from the samples and transferred to petriplates containing 2 different cultural media: 2% malt extract agar and PDA medium amended with 250 µg Streptomycin sulphate per ml. The first medium was intended to isolate Basidiomycetous fungi and the second medium to specifically isolate total fungi. Eight pieces of wood were removed from each sample and placed in 2 petriplates. These plates were incubated at 25±2 °C for 7 days. Once fungal colonies were formed in the agar plates, each colony was transferred to a new agar slant to obtain a pure culture.

3. CULTURAL CHARACTERS

For cultural studies Petri dishes of (100 mm outer diameter, containing 20 ml agar) were inoculated with a piece of mycelium at the edge and were kept in diffused daylight at room temperature (25±2 °C) and examined at 7 days intervals. Separate slides were prepared from the marginal region, the aerial mycelium and the submerged mycelium; they were mounted in lactophenol with cotton blue, which gives a cyanophilic reaction with certain structures.

Diagnostic Characters

Chemical tests: A rapid plate assay method was used to determine the presence of laccase, peroxidase, and catalase producing isolates. One to two weeks old mycelial growth on MEA plates was flooded with 25 mM guaiacol in 5 mM KHPO₄ pH 6, to determine the presence of laccase. If no reaction occurred, the plate was then flooded again with 2 mM H₂O₂ to determine the presence of peroxidase (Garraway *et al.* 1989, Harkin and Obst, 1973). To determine the presence of catalase activity, approximately 0.5 ml of 3% hydrogen peroxide was placed on a 1-3 week old colony on a MEA plates. The evolution of continuous bubbling was examined immediately and then again after a few min for positive reaction (Smibert and Krieg, 1981). One drop 4% KOH was placed on the aerial mycelium and any colour change was noted. Also a mycelial preparation was made in water; while observing the hyphae under the microscope, the water was gradually replaced by 10% KOH. The most common reaction is a colour change from yellowish to reddish or purplish and from brown to dark purple or blackish.

Growth rate: The colony radius was measured after one and two weeks. The growth rate used in the key is the colony radius after two weeks.

Characters of the Fungal mat

Advancing zone: At the very margin of the colony the medium can be submerged (Growing in the agar), appressed (Prostrate on the agar surface) or raised. The hyphae may grow so densely that the individual tips cannot be seen with the naked eye when the plate is held against the light or they may be so distant as to appear fimbriate. The outline of the margin may be even but is sometimes fringed or bayed.

Aerial mycelium: The texture of the mycelial mat is described by one or more of the following terms like absent, downy, farinaceous, granular, silky, cottony, woolly, floccose, plumose, pellicular or subfelty, velvety, crustose, and zonate (Stalpers, 1978).

Colony colour: Seven large groups have been distinguished. Colony of a fungus may be (a) uncoloured, (b) white, (c) cream, (d) yellowish or ochraceous, (e) brownish, (f) orange or reddish and (g) pink, pale lilac, blue, vinaceous or violaceous.

Other macroscopic characters

Odours: Attention has been paid to odours which show very distinct and definable odours in culture.

Reverse: The term reverse is used here for colour change in the agar underneath mycelium, induced by the fungus. It may be unchanged, bleached or darkened.

Characters of hyphae

Clamps: The distribution of the clamps is generally considered as having major taxonomic importance in Basidiomycetes members. It is constant within a species and generally also within a genus unless the fungus returns to the haploid condition. Four groups can be recognized 1) Clamps present at all septa, 2) Clamps absent or rare in the advancing zone, but present at nearly all other septa, 3) Clamps of erratic or rare occurrence, when present usually only at the border hyphae. There may be more than one clamp at each septum, 4) Clamps absent.

Hyphal width: Five intervals of hyphal width have been assessed: (a) hyphae $\leq 1.5 \mu\text{m}$, (b) Hyphae $1.5-3 \mu\text{m}$ (c) hyphae $3-5 \mu\text{m}$ (d) hyphae $5-7.5 \mu\text{m}$ and (e) hyphae $\geq 7.5 \mu\text{m}$.

Differentiation of the hyphae: 1) Generative hyphae: septate, branched, thin to thick-walled hyphae. For the thickness of the wall three categories are distinguished (a) thin walled ($0.2 \mu\text{m}$ or less), (b) firm walled ($0.2-0.3 \mu\text{m}$) and (c) thick-walled ($0.3 \mu\text{m}$ or more), 2) Skeletal hyphae: non-septate, unbranched or rarely branched, thick walled, straight or slightly flexuous., 3) Much-branched binding hyphae: non-septate, much branched, thick walled, strongly interwoven, typically narrow., 4) Hyphae with irregularly thickened walls, with meandering lumen or with thick walled, refractive areas inside the wall., 5) Hyphae with encrusted and usually contorted hyphal tips, usually erect and arising directly from the agar, giving the colony a mealy appearance., 6) Encrusted hyphae, occurring in many species in the aerial or submerged mycelium., 7) Hyphae covered with resinous material or oil drops, often yellowish or brownish. 8) Hyphae covered with minute projections which may be spiny or

blunt but consist of wall material. 9) Aerial hyphae containing oil drops or resinous material. 10) Much branched thin walled hyphae, often described as witches brooms can be observed in the marginal zone.

Setae: Thick walled, brown more or less pointed hyphae, typically rather short and with a basal median swelling. **Setal hyphae:** Long thick walled brown, pointed hyphae with or without some inconspicuous swellings. **Asterohyphidia:** Thick walled, brown hyphae with radiating branches. **Acanthohyphidia:** Clavate or cylindrical structures with pin like out growths. **Cystidia:** Terminal or rarely lateral, cylindrical, clavate or ovoid structures which are thin to thick walled hyaline or brownish in colour. **Gyoeocystidia:** Cylindrical, clavate or ovoid structures, terminal or more rarely, lateral, thin to firm walled, filled with refractive or resinous material, hyaline or yellowish. **Cuticular cells:** Terminal, lateral or intercalary swelling or complexes of swelling which form a pseudo-paranchymatous crust. Propagative structures like Chlamydospores, Blastoconidia, Arthroconidia, Conidiophores and Basidiospores were seen (Stalpers 1978).

4. RESULT AND DISCUSSION

Identification of isolates producing Volatile organic compounds

The 11 fungi associated with the wood samples were isolated. The growth of fungi was much better in Malt extract medium. They were identified as *Coriolus versicolor* (L.) Quél., *Flavodon flavus* (Klotz.) Ryv., *Ganoderma lucidum* (Curtis) P. Karst, *Hexagonia apiaria* (Pers.) Fr., *Lenzites betulina* (L.) Fr. *Lenzites eximia* Berk. & M.A. Curtis, *Lenzites sterioides* (Fr.) Ryv., *Navisporus floccosus* (Bres.) Ryv., *Phellinus gilvus* (Schwein.) Pat., *Schizophyllum commune* Fr., *Sterium hirsutum* (Willd.) Pers., and *Trametes pini* (Thore) Britzelm. In the present study all lignicolous fungi show white rot decay in woods. They belong to five orders and six families included in Class Agaricomycetes, Subclass Agaricomycotina, Phylum Basidiomycota (Table 1).

Table 1. list of lignicolous fungal showing white rot

Order	family	species	Type of rot
Polyporales	Polyporaceae	<i>Coriolus versicolor</i> (L.) Quél.	White rot
Polyporales	Meruliaceae	<i>Flavodon flavus</i> (Klotzsch) Ryvarden	White rot
Polyporales	Ganodermataceae	<i>Ganoderma lucidum</i> (Curtis) P. Karst	White rot
Polyporales	Polyporaceae	<i>Hexagonia apiaria</i> (Pers.) Fr.	White rot
Polyporales	Polyporaceae	<i>Lenzites betulina</i> (L.) Fr.	Brown rot

Polyporales	Polyporaceae	Lenzites eximia Berk. & M.A. Curtis,	White rot
Polyporales	Polyporaceae	Lenzites sterioides (Fr.) Ryv.	White rot
Polyporales	Polyporaceae	Navisporus floccosus (Bres.) Ryvardeen	White rot
Hymenochaetales	Hymenochaetaceae	Phellinus gilvus (Schwein.) Pat.,	White rot
Agaricales	Schizophyllaceae	Schizophyllum commune Fr.	White rot
Russulales	Stereaceae	Sterium hirsutum (Willd.) Pers.	White rot
Polyporales	Polyporaceae	Trametes pini (Thore) Britzelm.	White rot

Table 2. Cultural characters of lignicolous fungi with odour and volatile organic compound

species	Exudate	Reverse	odour	Volatile organic compound
Coriolus versicolor (L.) Quél.	cream	white	Woody	yes
Flavodon flavus (Klotzsch) Ryvardeen	cream	yellow	fruity	yes
Ganoderma lucidum(Curtis) P. Karst	cream	white	Rotten wood	yes
Hexagonia apiaria (Pers.) Fr.	no	cream	woody	yes
Lenzites betulina (L.) Fr.	colourless exudates	white	mushroomy	yes
Lenzites eximia Berk. & M.A. Curtis,	yellowish	cream	Woody	yes
Lenzites sterioides (Fr.) Ryv.	cream	Light yellow	woody	yes
Navisporus floccosus (Bres.) Ryvardeen	cream	white	Moist soil	yes
Phellinus gilvus (Schwein.) Pat.,	no	White to pale brown	Woody	yes

Schizophyllum commune Fr.	cream	unchanged	fruity	yes
Sterium hirsutum (Willd.) Pers.	no	cream to brown with dark brown areas	rotten wood	yes
Trametes pini (Thore) Britzelm.	no	white	moist soil	yes

Table 2, shows that Exudates were produced by 7 out of 11 species of lignicolous basidiomycetes i.e. *Coriolus versicolor* (L.) Quél. *Flavodon flavus* (Klotzsch) Ryvarden *Ganoderma lucidum*(Curtis) P. Karst *Lenzites betulina* (L.) Fr. *Lenzites eximia* Berk. & M.A. Curtis, *Lenzites sterioides* (Fr.) Ryv. *Navisporus floccosus* (Bres.) Ryvarden, *Schizophyllum commune* Fr. and Volatile organic compounds are synthesized by lignicolous fungi in pure cultures so the quantity and quality of the volatile organic compounds synthesized by lignicolous basidiomycetes indicate the presence of characteristic odours. The characteristic smell was identified for every species of lignicolous basidiomycetes that we isolated in pure culture. *Trametes pini* (Thore) Britzelm and *Navisporus floccosus* (Bres.) Ryvarden colonies shown moist soil fragrance, *Sterium hirsutum* (Willd.) Pers and *Ganoderma lucidum*(Curtis) P. Karst colonies shown rotten wood scent, *Schizophyllum commune* Fr. and *Flavodon flavus* (Klotzsch) Ryvarden colonies shown fruity aroma and *Coriolus versicolor* (L.) Quél. *Hexagonia apiaria* (Pers.) Fr. *Lenzites eximia* Berk. & M.A. Curtis, *Lenzites sterioides* (Fr.) Ryv. *Phellinus gilvus* (Schwein.) Pat., colonies shown woody scents (Table 2). All these odours are a clear indicator of the presence of volatile organic compounds produced by the lignicolous basidiomycetes grown on synthetic media. Suay and co-workers [2000] tested 317 fungal isolates belonging to 204 species of basidiomycetes and have proven that more than 45% of the isolates (over 109 species) have antibacterial and antifungal properties because of presence of volatile organic compounds. So in the present study also 11 lignicolous fungi were able to produce volatile organic compounds which may be have antibacterial and antifungal properties.

Macroscopic and microscopic characters of mycelium

Coriolus versicolor

On the Malt extract agar plates with tannic acid it showed a positive reaction for oxidases and Laccase, catalase/tyrosinase negative, peroxidase positive, In KOH it turns brown, Growth characters: Growth rapid, plates covered in two weeks >70 mm. Advancing zone even, dense, hyaline, appressed for short distance in advance of aerial' mycelium. Mat white, raised cottony-woolly, otherwise felty, mycelium grown up sides of Petri dish and across cover after three weeks. Reverse bleached. Odour is present. Growth of colony is 1.5 cm. diameter on Gallic acid agar and 2.5 - 3.0 cm diameter on tannic acid agar.

Hyphal characters: Advancing zone: hyphae hyaline, nodose-septate, with clamp, 3.12 - 6.25 μ diameter. Aerial mycelium: (a) hyphae as in advancing zone; (b) fiber hyphae numerous, thick-walled, lumina discernible only at bases of branches, frequently branched, 2.0-3.12 μ m diameter, curving and interwoven; (c) chlamydospores fairly numerous,

sometimes arthroconidium like, usually found lying free in preparations for microscopic examination, thin-walled, $4.5 - 9.33 \times 3.12 - 6.25 \mu\text{m}$. Submerged mycelium: hyphae as in advancing zone without clamps. Cosmopolitan.

Flavodon flavus

On the MEA medium containing tannic acid, it showed positive oxidase reactions for, laccase strongly, tyrosinase and peroxidase. In KOH, turned to dark brown, Growth characters: growth rate was $>70 \text{ mm}$ in 7 d, marginal hyphae were raised, colony even, aerial mycelium silky, colony colour cream, azonate,. The mycelial mat was cream coloured and evenly covered in the central portion of the inoculated plate. It become raised towards margin and thick with yellow colour. The surface was filamentous or fibrous. On the inoculated wooden blocks, the mycelium appeared fibrous. At a later stage at the margin of the plate, the mycelium appeared brown. At an advanced stage, the surface and margin appeared guttulate with yellow orange oil like globules. These were secretions released from fungal hyphae. Odour present, reverse bleached

Hyphal characters: Advancing zone: hyphae hyaline, nodose-septate, with clamp, $3.12 - 7.25 \mu$ diameter. Aerial mycelium: (a) hyphae as in advancing zone; (b) fiber hyphae numerous, thick-walled, branched, $3.12 - 6.25 \mu\text{m}$ diameter; (c) chlamydospores numerous, sometimes arthroconidium, thin-walled, $4.5 - 9.33 \times 3.12 - 6.25 \mu\text{m}$. Submerged mycelium: hyphae as in advancing zone with clamps. Cosmopolitan.

Ganoderma lucidum

On Malt extract agar medium with tannic acid it showed positive reaction to oxidase and strong positive reaction to laccase, and Strong positive reaction to peroxidase. Negative reaction to catalase/tyrosinase. In KOH it turns to rusty brown. Growth characters: Growth rapid, plates covered in one weeks and covers 70 mm in one weak. Advancing zone even, raised aerial mycelium extending to limit of growth. Mat white, azonate, with color subsequently masked by overgrowth of whitish 'bloom', at first slightly raised, cottony, then appressed. Reverse unchanged for two to three weeks, then finally bleached. Odour present On tannic acid agar growth of colony was $2.0-4.0 \text{ cm}$ in diameter in seven days.

Hyphal characters. Advancing zone: hyphae hyaline, nodose-septate, with clamps, $3.12 - 5.5 (6.0) \mu\text{m}$ diameter. Aerial mycelium, thin tough skin that peels from agar (a) hyphae as in advancing zone, with frequent branches and numerous small projections; (b) fibrous hyphae very numerous, with walls thick and refractive, lumina narrow or apparently lacking, except in main hyphae, frequently branched, the ends long, slender, curving and interwoven, $1.0 - 3.12 \mu\text{m}$ diameter; (c) cuticular cells thin-walled, produced by inflation of nodose-septate hyphae, at first with contents staining in phloxine, then empty, closely compacted and interwoven with fiber hyphae and staghorn hyphae to form pseudoparenchymatous layer, which may remain hyaline or become brown; (d) chlamydospores numerous, walls slightly thickened, terminal and intercalary, broadly ovoid to elongate, $12.25 - 21.65 \times 6.25 - 12.5 \mu\text{m}$. Submerged mycelium: (a) nodose-septate hyphae without clamps and (b) chlamydospores as described above.

Hexagonia apiaria

On Malt extract agar medium with tannic acid it showed positive reaction to oxidase and strong positive reaction to laccase and positive reaction to peroxidase. Negative reaction to catalase/tyrosinase. In KOH it becomes brown. Growth characters: Growth rapid covered

petri plate in one week, Advancing Zone, even, dense, hyaline, areal mycelium is appressed. Mat white, Plumose, azonate, later changes to cinnamon brown. Reverse bleached. Odour present.

Hyphal characters: Advancing zone: hyphae hyaline, septate, with clamp, 3.12 μ in diameter. Aerial mycelium (a) hyphae as in advancing zone; (b) fibrous hyphae numerous, thick-walled, narrow lumen, branched, 3.12- 6.25 μ m diameter, curving and interwoven; (c) chlamydospores few, smooth, thin-walled, 3.12 - 6.25 \times 3.12 μ m.

Lenzites betulina

On Malt extract agar medium containing tannic acid showing strong positive reaction to oxidases, and laccase, positive reaction to peroxidase negative reaction to catalase/tyrosinase. In KOH it becomes pale brown. Growth characters. Growth moderately rapid, covers 25-40mm in two weeks. Whole plates covered in four weeks. Advancing zone even, hyaline distant, and appressed in narrow zone and slightly raised aerial mycelium extending to limit of growth. Mat white, the newest growth slightly raised, woolly, becoming felty to lacunose, often with balls of mycelium, Basidiocarps typically not formed, becoming patchy, very tough, cream coloured in older cultures, all peeling readily from agar, the mycelium frequently grown up sides and down between lid and base of Petri dish. Reverse bleached. Odour present. Colony growth on tannic acid agar is 2.5 - 4.5 cm. in diameter in seven days.

Hyphal characters. Advancing zone: hyphae hyaline, nodose-septate, 3.2 - 5.5 μ m diameter. Aerial mycelium: (a) hyphae as in advancing zone; (b) fibrous hyphae very numerous, thick-walled and refractive, narrow lumen, much branched, 3.12 - 6.25 μ m diameter. Submerged mycelium: (a) nodose-septate hyphae with clamps and (b) fiber hyphae as in aerial mycelium. Cosmopolitan.

A soft cottony mycelial ring is formed on the walls. Submerged mycelium from the advancing zone presents generative hyphae, branched, septate, with clamp connections, (Balaeş and Tănase 2012b) but in the present study no mycelia ring formation and nodose-septate hyphae have clamps.

Lenzites exima

On Malt extract agar medium containing tannic acid showing strong positive reaction to oxidases, and laccase, positive reaction to peroxidase, negative reaction to catalase/tyrosinase. In KOH it becomes brown. Growth characters. Growth moderately rapid, covers 20-40mm in two weeks. Whole plates covered in 3 weeks. Advancing zone even, hyaline distant, and appressed in narrow zone and slightly raised aerial mycelium extending to limit of growth. Mat white, the newest growth slightly raised, woolly, becoming felty to lacunose, often with balls of mycelium, Basidiocarps typically not formed, becoming patchy, very tough, cream colors in old cultures, all peeling readily from agar. Reverse bleached. Odour absent. Colony growth on tannic acid agar is 3.5- 5.5 cm. in diameter in seven days.

Hyphal characters. Advancing zone: hyphae hyaline, nodose-septate, 3.2-6.5 μ m diameter. Aerial mycelium: (a) hyphae as in advancing zone; (b) fibrous hyphae very numerous, thick-walled and refractive, narrow lumen, branched, 3.12- 7.25 μ m diameter. Submerged mycelium: (a) nodose-septate hyphae without clamps and (b) fiber hyphae as in aerial mycelium. Cosmopolitan.

Lenzites sterioides

On Malt extract agar medium containing tannic acid showing strong positive reaction to oxidases, and positive reaction to laccase and peroxidase. Negative reaction to catalase/tyrosinase. In KOH it becomes pale brown. Growth characters: Growth rapid, covers petri plate with in two – four weeks. Advancing Zone, wavy some times and mostly even, densed, hyaline, areal mycelium is raised for some distant and appressed as it becomes old. Mat white, cottony-wooly, becomes locally floccose, azonate, later changes to creamy white, thin layer of mycelium balls pealed from the agar. Reverse unchanges. Odour present.

Hyphal characters: Advancing zone: hyphae hyaline, mostly septate, with clamp, 1.2 - 3.12 μ in diameter. Aerial mycelium (a) hyphae as in advancing zone, thin walled, branched, wider lumen with 6.25 μ m in diameter; (b) fibrous hyphae thick-walled, narrow lumen, branched, 3.12 - 6.25 μ m diameter; (c) chlamydo spores numerous, ellipsoidal, smooth, thin-walled, 6.25 - 12.52 \times 3.12 - 6.25 μ m. Submerged mycelium: hyphae as in advancing zone without clamps. Mostly on Angiosperms woods. Tropical

Navispora floccosus

On Malt extract agar medium with tannic acid it showed positive reaction to oxidase, laccase and peroxidase. Negative reaction to catalase/tyrosinase. In KOH it becomes pale brown. Growth characters: Growth rapid, covers petri plate in three week, covers >70mm in two weeks, Advancing zone wavy, densed, hyaline, areal mycelium is raises always. Mat white, cottony-wolly, azonate, later changes to cream colour. Reverse bleached. Odour present.

Hyphal characters: Advancing zone: hyphae pale yellow, septate, with clamp, 3.12 – 6.25 μ in diameter. Aerial mycelium (a) hyphae as in advancing zone; (b) fibrous hyphae numerous, yellowish in colour, thick-walled, narrow to wider lumen, branched, aseptate, 6.25 - 8.23 μ m diameter; (c) chlamydo spores few, ellipsoidal, thin-walled, 3.12 – 6.25 \times 3.12 μ m. Submerged mycelium: hyphae as in advancing zone without clamps.

Phellinus gilvus

On Malt extract agar medium with tannic acid it showed positive reaction to oxidase, laccase and negative to peroxidase and catalase/tyrosinase. In KOH it becomes brown. Growth characters: Growth rapid, covers petri plate in four week, Mycelium presents concentric zones, with abundant aerial hyphae, cottony, cream-brown. Forms brown crusts around the edges, and a brown ring with numerous aerial hyphae, high, tangled, cream to brown. Mycelium mat is felty, but very heavy, felty cottony, orange on the edges. The crusts are powdery, sometimes with small clusters of aerial hyphae. Reverse bleached. Odour present.

Hyphal characters: Advancing zone Submerged mycelium and from the advancing zone presents generative hyphae, thin, branched, with simple septa and rare, 3.12 to 6.25 μ m thick, long. Aerial mycelium presents: generative hyphae and thick skeletal hyphae, with few septa, pigmented, sometimes inlaid.

Schizophyllum commune

On the malt agar plates containing tannic acid, it showed a positive reaction for oxidase and laccase and negative reaction for tyrosinase. Peroxidase test was positive with growth rate of 20 to 40 mm in 14 d. In KOH, it turned to brown colour, marginal hyphae raised, and

distant of marginal hyphal tips dense. The outline of colony was white, odour absent, reverse of plate was darkened, clamps present, hyphae thin walled, septate, hyaline and 2 x 3.125 mm thick. The mycelia mat was purely white in colour initiating near the inoculum and spreading throughout the surface of the medium. The surface appears to be curled or folded. It showed a smooth and dull texture. odour present

Hyphal characters: Advancing zone: hyphae hyaline, mostly septate, with clamp, 3.12 - 6.25 μ in diameter. Aerial mycelium (a) hyphae as in advancing zone, thin walled, branched, wider lumen with 6.25 μ in diameter; (b) fibrous hyphae thick-walled, narrow lumen, branched, 3.12 μ diameter; (c) chlamydospores numerous, elliptical, smooth, thin-walled, 6.25 - 12.52 \times 3.12 - 6.25 μ m. Submerged mycelium: hyphae as in advancing zone without clamps. Mostly on Angiosperms woods. Cosmopolitan

The mycelium formed after three weeks several fruiting bodies between the two plates, but also in the middle of the colony, reaching the upper plate. As described by Balaeş and Tănase (2012a), on all media, several hyaline chlamydospores, of 9 – 15 \times 4 – 5 μ m were produced by this species. But in the present paper also chlamydospores and primordial development in the middle of petri plate were reported

Sterium hirsutum

On Malt extract agar medium with tannic acid it showed negative reaction to oxidase, laccase and peroxidase and positive reaction to catalase/tyrosinase. In KOH it becomes pale yellow to red. Growth characters: Growth very rapid, covers petri plate in two week, covers >70mm in one weeks, Advancing zone even, densed, hyaline, areal mycelium is raises for some distance and becomes appressed as culture becomes old. Mat creamy, plumose, azonate, later changes to creamish yellow. Reverse bleached. Odour present.

Hyphal characters: Advancing zone: hyphae pale yellow to hyaline, septate, multiple clamp, branched, 3.12 – 12.56 μ in diameter. Aerial mycelium (a) hyphae as in advancing zone; (b) fibrous hyphae numerous, yellowish in colour, thick-walled, narrow to wider lumen, branched, aseptate, 3.125 - 9.33 μ m diameter; (c) chlamydospores absent. Submerged mycelium: hyphae as in advancing zone without clamps. On various Angiosperms woods. Cosmopolitans.

Stereum hirsutum

(Willd.) Pers. On MEA and PDA the colony is cream colored with light orange areas, heterogeneous, with downy areas where the hyphae tend to crowd, giving the colony a granular aspect, alternating with translucent areas where the submerged mycelium is seen. The edge of the colony is irregular After four weeks of incubation, the colony forms on the edges of the plate, near the upper part of the Petri dish primordial of fruiting bodies (Virginia and Cătălin 2013)

Trametes pini

On Malt extract agar medium with tannic acid it showed positive reaction to oxidase, and laccase and Negative reaction to peroxidase and catalase/tyrosinase. In KOH it becomes rusty brown. Growth characters: Growth rapid, covers Petri plate in three weeks. Advancing zone even, dense, white, raised aerial mycelium extending to limit of growth later it appressed. Mat white, pale yellow in old culture raised, azonate, cottony woolly to woolly, tufted to form a rough surface. Reverse less colored appears brown, later it was darker. Odour was present.

Hyphal characters. Advancing zone: hyphae hyaline, with simple septa, having few clamps 3.12-5.5 μm diameter. Aerial mycelium: (a) hyphae as in advancing zone; (b) hyphae with slightly thicker walls, with contents yellow, brown, septate, branched, 3.12-6.25 μm diameter, characteristically with scattered dark brown cells in hyaline or pale hyphae, frequently helicoid; (c) expansions on hyphae up to 8.5 μm diameter chlamidospores in a terminal or intercalary position, usually with walls brown and thickened; (d) setae numerous in some isolates, rare, slender, pointed, with walls thick and dark brown, 30.0-67.0 x 6.5-9.33 μm .

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ILLUSTRATION OF FIGURES

Plate I

Fig. A: culture of *Schizophyllum commune*

Fig. B: culture of *Schizophyllum commune* showing primordial

Fig. C: culture of *S. Commune* Showing upper surface of fruiting body

Fig. D: culture of *S. Commune* Showing lower surface of fruiting body

Fig. E. Culture of *Lenzites sterioides*

Fig. F. Culture of *Phelinus gilvus*

Plate II

Fig. A. Culture of *L. Sterioides* showing fruiting body

Fig. B. Culture of *L. Sterioides* showing mature Basidiocarp

Fig. C. Culture of *Flavodon flavus* showing primordial

Fig. D. Culture of *Trametes pini*

Fig. E. Culture of *Lenzites betulina*

Fig. F. Culture of *L. eximia*

Plate I

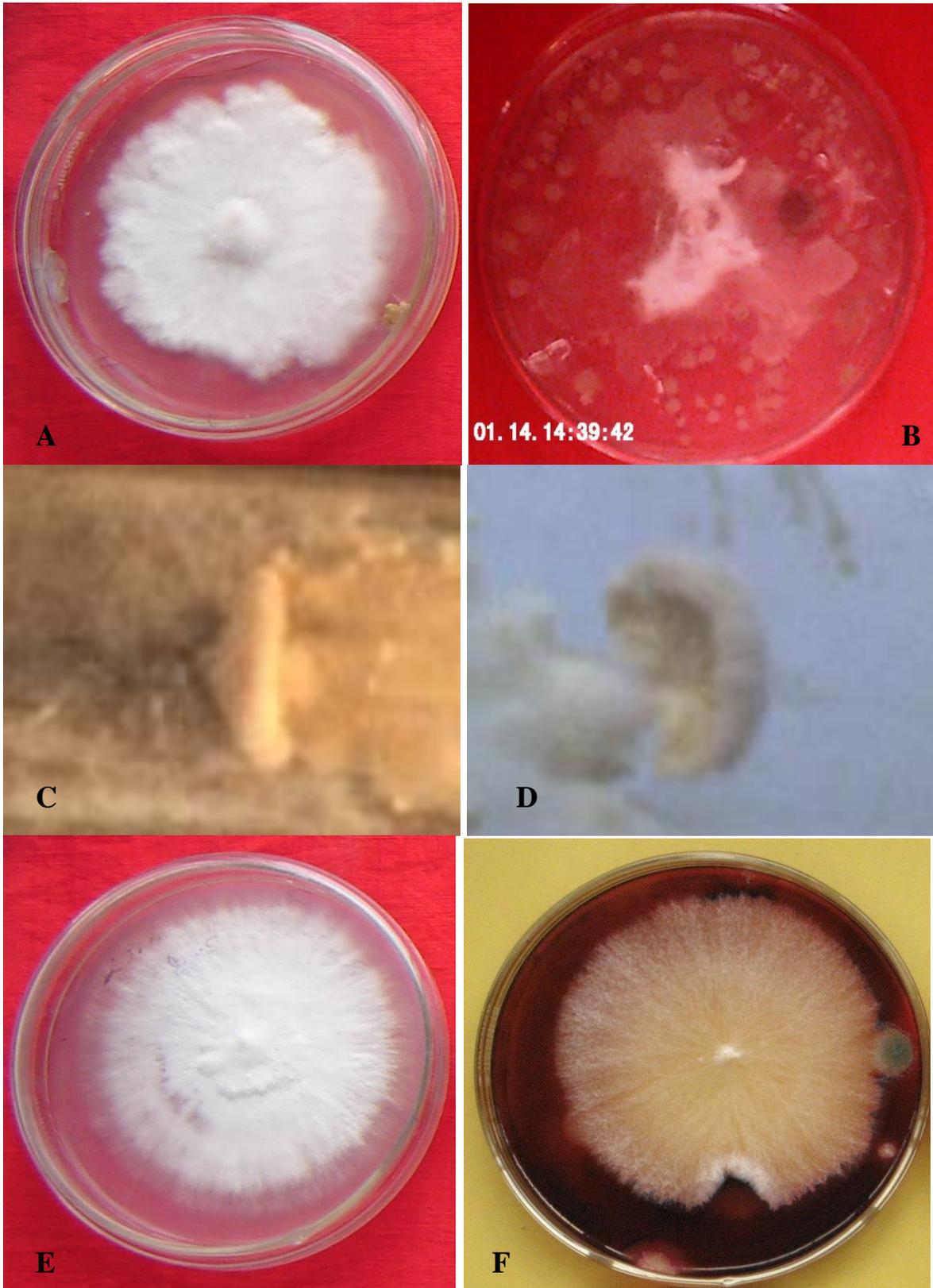


Plate II

