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Mycosphere Essay 11: Fungi of *Pycnoporus*: morphological and molecular identification, worldwide distribution and biotechnological potential

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Abstract

Fungi of the *Pycnoporus* are efficient degraders of lignocellulosic materials, so they are classified as white-rot fungi. A distinctive feature is its color ranging from orange to bright red, attributed to cinnabarin, cinnabarinic acid and tramesanguin mainly, compounds to which have been attributed some biological activities. This review updates the reports on morphological characteristics, the distribution of species of *Pycnoporus*, and the advantages of molecular techniques in species identification. Furthermore, the potential biotechnological applications of these fungi are mentioned with a focus on insecticidal and antibacterial activities. In addition, the production of hydrolytic enzymes including cellulases, hemicellulases, pectinases are discussed as well as phenoloxidases such as laccases and their participation in the degradation of agro-industrial waste.

Keywords – Biotechnological applications – *Pycnoporus sanguineus* – *Pycnoporus cinnabarinus* – taxonomy – molecular identification

Introduction

Correct identification of fungi is of great importance. A huge number of fungal species have been described, but even taxonomists are kept busy with the recognition and description of new species and taxa. Therefore, fungal species identification based on morphological characteristics, origin and molecular biology approaches has been limited in some countries and for unskilled persons, it can be a challenge (Guarro et al. 1999). The most important characteristics used in general on the identification of fungi are: 1) Shape of fruiting body, 2) Color of each of the parts of the fruiting body, including internal (meat) and the underground part, 3) Some structures or characteristics of the fruiting body such as: flakes, warts, hairs, spines, pores, stretch marks,

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viscosity, and meatiness, among others, 4) Change in color when manipulated or cut anywhere in the fruiting body, 5) Presence or absence of a milky juice or latex when cut, the latex color should be checked before and after its exposure to air, 6) Smell of fungus (mainly meat), 7) Flavor of the meat, 8) Color of the mass spore and 9) The identity or type of host on which the fruiting body is located (Guzmán 1979). To date, the description and exploration of the diversity of *Pycnoporus* has been based primarily on the morphological similarity of the specimens referred to with classified specimens in international collections, even though the identification remains a challenge due to the high variability of morphological characteristics during growth. Morphological characteristics of young specimen vary depending on the fruiting body age, kind of wood where they grow, and environmental factorssuch as, temperature, humidity and/or light.

General description of *Pycnoporus* genus

The genus *Pycnoporus* is a cosmopolitan group of white rot fungi, belonging to the phylum Basidiomycota, Agaricomycetes class, Polyporaceos order (Ryvarden 1978, Hibbett et al. 2007). It is related with the genus *Trametes*, morphologically it is similar in all characteristics except for the bright red-orange color of their basidiocarps (Ryvarden 1991, Ryvarden & Gilbertson 1994). The *Pycnoporus* fungi are heterothallic homobasidiomycetes with a tetrapolar mating system (Nobles & Frew 1962). *Pycnoporus* fruiting body tends to show the greatest morphological variation, such as size, shape, color, surface texture and consistency, hyphal system, pores, tubes, and macro-chemical reactions, for instance, color changes when sample is mistreated, dry or exposed to 5% KOH, Melzer solution and phenol (Cifuentes et al. 1986, Gilbertson & Ryvarden 1986). This genus has annual fruiting, and characteristics include smooth pileus, sessile and coriaceous, shelf shaped halfmoon and sometimes faintly marked concentric zones or not. In mature specimens this surface is a whitish or silvery colour (Guzmán 2003). The underside has isodiametric pores ranging in size from small to medium. The context is of the same color as the pileus, and is thin to moderately thick. This genus is characterized by a dimitic or trimitic hyphal system with generative hyphae of thin walls, hyalines and septate, tortuously united with skeletal hyphae of thick walls. Skeletal hyphae present with clear pinkish coloring in preparations. Spores are cylindrical, and hyalines have thin walls, and are smooth and amyloid. Another technique used to verify the identity of the species involves cultivation of different spores. If binding is detected between two strains, then they are considered identical and if not they are different species (Deacon 2013). Table 1 shows the nine *Pycnoporus* species and their synonymous for the same taxon reported in the Index Fungorum "virtual page, which is an international project to index all scientific names in the Fungi kingdom, it is comparable to International Plant Names Index" (Ulloa & Hanlin 2006).

Historically, four species were differentiated based on their morphology (basidiocarp shape and size of their pores) and their distribution area (Nobles & Frew 1962, Ryvarden & Johansen 1980), although are very similar, there is a high degree of difficulty to identify wild or cultivated specimens (Lesage-Meessen et al. 2011). For *Pycnoporus sanguineus*, *Pycnoporus cinnabarinus*, *Pycnoporus coccineus* and *Pycnoporus puniceus* the following taxonomic data have been descry-bed, and in Table 2 the main basidiocarp morphological differences among them are pointed out.

Pycnoporus sanguineus (L.) Murril

Annual basidiocarp, pileus shortly and sessile or sometimes overlapping, solitary to gregarious habitat, leathery, coriaceous to corky consistency, with a size 30–30 × 22-30 × 5–10 mm, diameter, width and thickness respectively. Semicircular pileus of dimidiate, flabelliforme, bright orange red when young and only reddish orange when ripe, velutinous surface to glabrous, zoned; acute margin, from smooth to wavy thin, sterile. Hymenophore poroide, color reddish orange; pores 4-6 per mm, circular tubes concolor to hymenophore, some specimens are concolor to the pileus, up to 1 mm deep, corky consistency to floccose (Fig. 1). It grows on dead wood, always sunny places and causes white rot. Macro-chemical reactions occur by adding 5% KOH resulting in a quick colour change to black in all its parts, but upon drying becomes greenish brown, so it gives a false xanthochromia reaction.

Table 1 Names of species of genus *Pycnoporus*.

	***Pycnoporus* specie**	**Synonymous**
1	*Pycnoporus cinnabarinus* (Jacq.) P. Karst (1881)	*Pycnoporus coccineus* (Fr.) Bondartsev & Singer (1941)
2	*Pycnoporus sanguineus* (L.) Murril (1904)	*Boletus ruber* Lam., (1783), *Boletus sanguineus* L., (1763), *Coriolus sanguineus (L.) G. Cunn., (1949)*, *Fabisporus sanguineus* (L.) Zmitr., (2001), *Microporus sanguineus* (L.) Pat., (1900), *Polyporus sanguineus* (L.)Fr., (1821), *Polystictus sanguineus* (L.) G. Mey., (1818), *Trametes cinnabarina var. sanguinea* (L.) Pilát, (1936), *Trametes sanguinea* (L.)Imazeki, (1943), *Trametes sanguinea (*L.)Lloyd, (1924).
3	*Pycnoporus coccineus* (Fr.) Bondartsev & Singer (1941)	*Polyporus coccineus* Fr. (1855) *Fomes coccineus* (Fr.) Sacc. (1888) *Scindalma coccineus* (Fr.) Kuntze (1898) *Polystictus coccineus* (Fr.) Lloyd (1916)
4	*Pycnoporus puniceus* (Fr.) Ryvarden (1972)	*Trametes punicea* Fr. (1851)
5	*Pycnoporus annosus* (Fr.) Karst (1898)	*Heterobasidium annosum* (Fr) Bref. (1888)
6	*Pycnoporus epilobii* (P. Karst) P. Karst (1889)	*Datronia steroids* (Fr.) Ryvarden (1967)
7	*Pycnoporus mimicus* P. Karst (1906)	*Fomitopsis cajanderi* (P. Karst) Kotl & Pouzar (1957)
8	*Pycnoporus palibini* P. Karst (1911)	
9	*Pycnoporus serialis* (Fr.) P. Karst (1889)	*Antrodia serialis* (Fr) Donk (1966)

Index Fungorum (www.indexfungorum.org).

Microscopic data: trimitic hyphal system, cylindrical spores of 5–6 × 2–3 µm, smooth, hyaline, non-amyloid, thin-walled (Table 2). Remarks: This species is characterized by sessile basidiocarps, dimidiate and smooth, with characteristic color of bright reddish orange, which becomes clearer when ripe, and by the pore size and its short tubes. The closest species is *Pycnoporus cinnabarinus*, which has thicker basidiocarps and whose coloration remains intense in the pores (Valenzuela 1999, Pompa et al. 2011). Habitat: It grows on dead wood and causes white rot. It has been reported in all tropical and subtropical areas of the world, mainly in places altered by man.

Fig. 1 – Fruiting body of *Pycnoporus sanguineus*, a) pileus, b) hymenophore.

Pycnoporus cinnabarinus (Jacq.) P. Karst

Annual basidiocarps, sessile, habitat solitary to gregarious, of consistency coriaceous to corky, with a size of 70 × 130 × 140 mm (diameter, width and thickness respectively). Pileus is dimidiate or elongated, of orange ocher color to pale orange. The color is intense when the specimen is growing, mature or dry specimens are pale and degraded specimens are whitish. The surface of the pileus when young is finely pubescent and it changes quickly to smooth or warty, sometimes in a reticulate pattern, with or without weakly ridged areas, acute margin, smooth to thin wavy, sterile. Context of 10-15 mm, tough and fibrous, usually the color is more persistent in degraded specimens. Hymenophore with pores, coral color to red; angular pores (2-3 per mm);

concolor tubes to the pileus when young of up to 7 mm depth (Fig. 2). It grows on dead wood, preferably on *Betula, Sorbus aucuparia, Alnus, Corylus, Fraxinus, Malus, Populus, Prunus, Quercus* y *Salix*, causing white rot. Macro-chemical reactions: By adding KOH, the colour quickly changes to black in all its parts.

Microscopic data: trimitic hyphal system with generative hyphae of thin wall, hyaline with fibulae of 1.5-3.5 microns wide, the skeletal hyphae are hyaline, cylindrical spores slightly bent of 5–6 × 2–3 µm, smooth, hyaline and non-amyloid (Table 2). Remarks: The red-orange fruiting bodies and smooth pileus are reliable features to confirm their identification. Due to their striking coloration these are frequently collected and this species is over-represented in herbaria compared with other species (Ryvarden 1978). This species is commonly reported in parts of the northern hemisphere. It is suggested that the presence of *Pycnoporus sanguineus* in Nordic locations may indicate some tropical reminiscence in the geological past (Guzmán 2003).

Fig. 2 – Fruiting body of *Pycnoporus cinnabarinus*, a) pileus, b) hymenophore.

Pycnoporus coccineus (Fr.) Bond & Sing.

Annual carpophores, sessile, its habitat is solitary to gregarious, coriaceous consistency to corky, varies from 1-2 cm in diameter in young specimens and 5 × 15 × 1 cm (diameter, width and thickness) in mature specimens. Pileus usually dimidiate, orange color when young and when mature becomes reddish orange. No top surface areas, velvety texture in young specimens and become hard and smooth with age. Obtuse margin, concolor with the upper surface. Context is corky pale of 3-10 mm thick, hymenophore with pores (Table 2), concolor to pileus and sometimes intense orange yellow, dark reddish-orange or bright red; 6-8 pores per mm, concolor tubes to hymenophore in some specimens, concolor to the pileus of 1.0 to 2.5 mm deep, cottony consistency to floccose (Fig. 3) (Nobles & Frew 1962). It grows on dead wood, and causes white rot. Macro-chemical reactions: By adding KOH, rapidly changes colour to black.

Microscopic data: trimitic hyphal system, with generative hyphae thin-walled and hyaline. Hyaline skeletal hyphae, the spores are cylindrical to slightly flattened, slightly curved 4.0–5.2 × 2.0–2.3 µm (diameter, width and thickness), smooth, hyaline, non-amiloyd. Remarks: It is suggested that specimens identified as *Pycnoporus coccineus* are young specimens or are actively growing of *Pycnoporus sanguineus* (Ryvarden & Johansen 1980). This species has been reported in countries bordering the Pacific Ocean and Indic.

Fig. 3 – Fruiting body of *Pycnoporus coccineus*, a) pileus, b) hymenophore.

Pycnoporus puniceus (Fr.) Ryvarden

Grows on deciduous wood, it is a rare species known from Africa (Angola, Ghana, Nigeria, Zaire), Asia (India, Malaysia) and Oceania (New Caledonia) (Ryvarden & Johansen 1980), and characterized by a basidiocarp with large irregular pores (1–3 per mm) (Lesage-Meessen et al. 2011). Microscopic data: trimitic hyphal system (Table 2), thick-walled, hyaline to yellowish, sparsely clamped, about 2.5–4 µm in diameter, yellowish skeletal (Fig. 4).

Fig. 4 – Fruiting body of *Pycnoporus puniceus*, a) pileus, b) hymenophore

Table 2 Main basidiocarp morphological differences among *Pycnoporus* species.

Characteristics	***Pycnoporus sanguineus***	***Pycnoporus cinnabarinus***	***Pycnoporus coccineus***	***Pycnoporus puccineus***
Basidiocarp	Smooth, flabelliforme velutinous surface to glabrous, zoned; acute margin, smooth to wavy thin, sterile.	Smooth or warty, sometimes in a reticulate patter, with or without weakly ridged areas, acute, smooth to thin margin, sterile	Hard smooth, coriaceous consistency to corky, 1 -2 cm Obtuse margin	Wrinkled, broadly attached
Color	Bright reddish orange	Orange-ocher to pale orange	Reddish orange	Dark orange innabar to darker red, with darker and lighter zones ending in the pore layer reflecting developmental stages
Size (mm)	30–30 × 22–30 × 5–10	70 × 130 × 140	50 × 150 × 10	40–50 × 13 × 7–10
Context	2 mm, corky consistency to floccose	10–15 mm, tough and fibrous	3–10 mm, corcky pale	Cinnabar to darker red, with darker and lighter zones ending in the pore layer reflecting developmental stages.
Hymenophore	Orange-red, pores 4–6 mm, tubes 1 mm deep	Coral to red, 2–3 angular pores per mm, con color tubes 7 mm deep coriaceous to corky mature or dry are pale and whitish in degraded specimens	Reddish orange sometimes intense orange yellow or bright red, 6–8 pores per mm, tubes 1.0–2.5 mm deep, cottony consistency to fluccose	Large pores 1–3 per mm
5% KOH	Upon drying becomes greenish brown	Changing to black	Changing to black	Changing to black
Hyphal system	Trimitic	Trimitic	Trimitic	Trimitic
Spores	Cylindrical spores 5–6 × 2–3 mm deep	Cylindrical to slightly bend spores 5–6 × 2–3 mm deep	Cylindrical to slightly flattened, slightly curve 4.0–5.2 × 2.0–2.3	Ellipsoid, thin-walled and with smooth walls, 4.5–6 × 23 µm.

(Ryvarden & Johansen 1980)

Molecular identification

In recent years, the most accurate assessment of the identification and diversity of fungi has been achieved using molecular markers, such as, the internal transcribed spacer (ITS) region of ribosomal DNA, and partial sequences of genes encoding subunits of RNA, such as polymerase, β-tubulin, cellobiohydrolase-C, topoisomerase II and Manganese peroxidase (Hatsch et al. 2004, Wang et al. 2004, Froslev et al. 2005, Tomšovský et al. 2006). There are other techniques used for molecular identification such as RFLP (restriction fragment length polymorphism) and RAPDs (random amplified polymorphic DNA). Ribosomal gene amplification is used to identify microorganisms (White et al. 1990, Dupont et al. 1998). These genes comprise highly conserved and highly variable sequences and can be used to compare species within the same genus phylogenetically and between strains of the same species. The region of nuclear rDNA contains two internal transcribed spacers regions (ITS1 and ITS2) and the gene 5.8S rDNA is used successfully in the determination of phylogenetic relationships among genera and species of fungi. The ITS region is known to show some variability even within species, although their intraspecific variability is often considered limited and clearly separated from interspecific variability. The existence of such a large variability between intra- and interspecific gap is implicitly assumed by automated approaches to species identification, but if indeed intraspecific variability is insignificant in the fungi kingdom, its use remains controversial (Nilsson et al. 2008).

This region can easily be amplified (600-800 bp sequence, which is repeated several times in the genome) with the PCR method using universal primers ITS1 and ITS4. Lomascolo et al. (2002) performed the molecular identification using ribosomal ITS1–5.8S-ITS2 of genomic sequences of 20 strains of *Pycnoporus* (2 of *Pycnoporus coccineus*, 8 of *Pycnoporus cinnabarinus* and 10 of *Pycnoporus sanguineus*), the phylogenetic tree of the sequences obtained grouped the strains according to their geographical origin rather than their taxonomic position. One group included the Asian strains and the other included northern Europe strains. Rungjindamai et al. (2008) performed the molecular characterization (ITS) of basidiomycetes from Thailand, and the species were identified as *Pycnoporus puniceus*, who formed a group with *Pycnoporus sanguineus*, grouped in a basal clade. *Pycnoporus puniceus* is a rare species. This is the first report of this species for Thailand. Ryvarden & Johansen (1980) have already reported its finding in Malaysia.

Phylogenetic trees inferred from sequences clearly show the difference in the group of strains of *Pycnoporus cinnabarinus* with the group of strains of *Pycnoporus puniceus*, strongly confirmed in clades (100%). The molecular grouping, based in sequences *lac 3-1*, separated the species by distribution in *Pycnoporus sanguineus* and *Pycnoporus coccineus* in four clades and subclades. A neotropical sub-clade, grouped strains of *Pycnoporus sanguineus* from France, Guiana and Venezuela, corresponding to *Pycnoporus sanguineus* sensu stricto. In a paleotropical subclade, strains from Madagascar, Vietnam and New Caledonia were pooled and defined as *Pycnoporus* cf. *sanguineus*. The clade of Australia corresponded to *Pycnoporus coccineus* in *stricto sensu*. The clade in the region of East Asia, and the grouping of strains from China and Japan, formed a group of *Pycnoporus coccineus*. The analysis of the laccase gene (*lac 3-1*) of *Pycnoporus* species highlighted functional diversity of the enzyme associated with the biogeographical origin (Lesage-Meessen et al. 2011).

Worldwide distribution

Fungi of the genus *Pycnoporus* are widely distributed. Some species have been reported in banks of strains (Table 3), and are well researched. The most studied species is *Pycnoporus sanguineus*, followed by *Pycnoporus cinnabarinus* then *Pycnoporus coccineus* and finally *Pycnoporus puniceus*. These species were described by Nobles & Frew (1962) and Ryvarden & Johansen (1980). *Pycnoporus sanguineus* and *Shizophyllum commune* have been considered indicators of disturbance species (Díaz-Barriga et al. 1988). Currently in Mexico there are specimens of the *Pycnoporus* genus in some mycological herbaria such as the Biological Research Center of the Universidad Autónoma del Estado de Morelos (HEMIM), MEXU collection of the Faculty of Sciences of the Universidad Nacional Autónoma de México (UNAM), Institute of

Biology of the UNAM, National School of Biological Sciences of IPN, University Center for Biological and Agricultural Sciences at the Universidad de Guadalajara.

Table 3 Distribution of some species of the genus *Pycnoporus*.

Specie	Strain	Host origen	Geographic origin	Reference
Pycnoporus puniceus	BCC26408	Saprobic on wood	Tammaran Pier, Satun	Rungjindamai et al. 2008
	BCC27595	Saprobic on wood	Tammaran Pier, Satun	Rungjindamai et al. 2008
	MUCL47083	Quercus cubana	Pinal del Rio, Cuba	Lesage-Meessen et al. 2011
	MUCL44170			Lesage-Meessen et al. 2011
	MUCL47087	Quercus cubana	Pinal del Rio, Cuba	Lesage-Meessen et al. 2011
	MUCL53064	Old timber, unidentified angiosperm	Europe, French Guiana, Regina Municipality, Les Nouragues Nature Reserve, CNRS field station Inselberg	Lesage-Meessen et al. 2011
Pycnoporus coccineus	CBS 355.63 (BRFM 65)	Wood	Solomon Islands	Lomascolo et al. 2002
	CBS 356.63	*Quercus serrata*	Japan	Lomascolo et al. 2002
	BRFM 937 (MUCL 38527)		Japan	Uzan et al. 2010
	BRFM 938 (MUCL 38523)	*Eucalyptus* sp.	Australia	Uzan et al. 2010
	BRFM 939 (MUCL 38525)	*Eucalyptus marginatus*	Australia	Uzan et al. 2010
	BCC22930	Bark of dead hardwood tree	Thailand	Thongkred et al. 2011
Pycnoporus cinnabarinus	MUCL 30555	Forest	Lauzelle forest, Belgium	Lomascolo et al. 2002
	MUCL 29375	Wood, ocean	Madagascar, Indian ocean	Lomascolo et al. 2002
	MUCL 38420	Nd	Australia	Lomascolo et al. 2002
	MUCL 38480	Nd	Austria	Lomascolo et al. 2002
	CBS 101046	Nd	Melbourne, Australia	Schliephake et al. 2000
	I-937 (CNCM)	Rotten wood	Finland	Lomascolo et al. 2002
	BRFM 44	Banque de Resources Fongiques de Marseille	Mar-seille, France	Alves et al. 2004
	F-076.859	Nd	Spain	Suay et al. 2000
	0261 (BIP)	Nd	Russia	Herpoël et al. 2000
	0263 (BIP)	Nd	Russia	Herpoël et al. 2000
	0692 (BIP)	Nd	Russia	Herpoël et al. 2000
	I-938 (CNCM)	Nd	France	Herpoël et al. 2000
	VKM F-3226	*Betula* sp.	RussiaChelyabinsk Region	Gómez-Toribio et. al. 2009

Specie	Strain	Host origen	Geographic origin	Reference
Pycnoporus sanguineus	F-057.299	Nd	Spain	Suay et al. 2000
	CBS 357.63	Pinus echinata	Georgia, USA	Halaouli et al. 2005
	SN	Nd	Kepong, Malaysia	Teoh et al. 2011
	SN	Parit Forest	Reserve, Perak, Malaysia.	Zulfadhly et al. 2001
	CBS 358.63	Shorea robusta	India	Lomascolo et al. 2002
	CBS 614.73	Angiosperm wood	Sri Lanka	Lomascolo et al. 2002
	G05	Rotten wood of broad-leaves tree	Guangdong: Mt. Dinghu, China	Lomascolo et al. 2002
	W006	Rotten wood of broad-leaves tree	Guangxi: Mt. Daming, China	Lomascolo et al. 2002
	006-2	Rotten wood of broad-leaves tree	Guangxi: Mt. Daming, China	Lomascolo et al. 2002
	W 3008	Rotten wood of broad-leaves tree	Guangxi: Mt. Daming, China	Lomascolo et al. 2002
	G53	Rotten wood of Pinus sp.	Hainan: Bawangling, China	Lomascolo et al. 2002
	G66	Rotten hardwood	Hainan: Tunchang, China	Lomascolo et al. 2002
	H2008	Rotten hardwood	Hainan: Bawangling, China	Lomascolo et al. 2002
	H2180	Rotten hardwood	Hainan: Qiongzhong, China	Lomascolo et al. 2002
	W28	Rotten wood of broad-leaved tree	Jiagdaqi, Mongolia, China	Halaouli et al. 2005
	G118	Rotten wood of broad-leaved tree	Heilongjiang, Huma, China	Halaouli et al. 2005
	Dis 343d	*Theobroma cacao*	Maldonado, Pichincha Province, west Ecuador	Crozier et al. 2006
	Dis 343f	*Theobroma cacao*	Maldonado, Pichincha Province, west Ecuador	Crozier et al. 2006
	Dis343c	*Theobroma cacao*	Maldonado, Pichincha Province, west Ecuador	Crozier et al. 2006
	BCC26410	Oil palm	Sai Bor oil plantation, Trang	Rungjindamai et al 2008
	BAFC 2126		Misiones Argentina	Shimizu et al. 2009
	BAFC: 2341		Misiones Argentina	Bobadilla et al. 2007
	SN		Uruguayan	Gioia et al. 2014
	BRFM 892	Nd	French Guiana	Uzan et al. 2010
	BRFM 893	Nd	French Guiana	Uzan et al. 2010
	BRFM 895	Nd	French Guiana	Uzan et al. 2010
	BRFM 896	Nd	French Guiana	Uzan et al. 2010
	BRFM 897	Nd	French Guiana	Uzan et al. 2010
	BRFM 899	Nd	French Guiana	Uzan et al. 2010
	BRFM 900	Nd	French Guiana	Uzan et al. 2010
	BRFM 901	Nd	French Guiana	Uzan et al. 2010
	BRFM 902	Nd	French Guiana	Uzan et al. 2010
	BRFM 903	Nd	French Guiana	Uzan et al. 2010
	BRFM 905	Nd	French Guiana	Uzan et al. 2010
	BRFM 906	Nd	French Guiana	Uzan et al. 2010
	BRFM 942	Nd	Vietnam	Uzan et al. 2010

Specie	Strain	Host origen	Geographic origin	Reference
	BRFM 943	Nd	Vietnam	Uzan et al. 2010
	BRFM 23	Nd	China	Uzan et al. 2010
	BRFM 3	Nd	China	Uzan et al. 2010
	BRFM 6	Nd	China	Uzan et al. 2010
	BRFM 11	Nd	China	Uzan et al. 2010
	BRFM 66	Nd	China	Uzan et al. 2010
	BRFM 881	rotten wood	Venezuela	Uzan et al. 2010
	BRFM 979	Nd	French New Caledonia	Uzan et al. 2010
	BRFM 980	Nd	French New Caledonia	Uzan et al. 2010
	BRFM 981	Nd	French New Caledonia	Uzan et al. 2010
	CCB175	Nd	São Paulo, Brazil	Rosa et al. 2003
	CCB113	Nd	São Paulo, Brazil	Rosa et al. 2003
	Bm	Nd	Mato Groso do Sul, Brazil	Rosa et al. 2003
	CCB273	Nd	São Paulo	Rosa et al. 2003
	CCB294	Nd	São Paulo	Rosa et al. 2003
	CCB277	Nd	São Paulo	Rosa et al. 2003
	CCT-4518	Fundação André Tosello, Campinas	São Paulo, Brazil	Garcia et al. 2007
	118775		Medellín (Antioquia), Colombia	Correa et al. 2005
	MEXU 25347	Oil-polluted	Veracruz, México	Dantán-González et al. 2008
	HEMIM 52, 52, 53, 54	Nd	Morelos México	Acosta-Urdapilleta et al. 2010

CNCM, Collection Nationale de Cultures de Microorganismes, Institut Pasteur, France; MUCL, Mycotheque de l'Universite Catholique de Louvain, Belgium; BIP, Botanical Institut of St Petersburg; BRFM, Banque de Ressources Fongiques de Marseille of the International Centre of Microbial Resources, Marseille, France; CBS, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; MEXU Herbario Nacional de México; Herbario Micologico de Morelos (HEMIM).

Biotechnological potential application

Medical & Pharmaceutical

As previously mentioned, *Pycnoporus cinnabarinus*, *Pycnoporus coccineus* and *Pycnoporus sanguineus* are able to synthesize a salmon red color pigment that sometimes can be described even as scarlet or vermillion which is a characteristic feature of the species. With the aging process the carpophore turns to an orange or pink color making it difficult in morphological characterization. Biological pigments or biochromes are substances produced by living organisms that have a color resulting from selective color absorption. Several compounds with structures of phenozaxone base are considered to be pigments known also as alkaloids or polyphenols. Most alkaloids have strong physiological effects in animals even at low doses so have psychoactive, medicinal and pharmaceutical applications. It has been reported that of the pigments of *Pycnoporus* strains; cinnabarin, cinnabarinic acid and tramesanguin are the main components, and act as antioxidants, free radical scavengers, antifungals (Borderes et al. 2011), anticarcinogens (Smânia et al. 2003), are immunomodulatory, larvicidal (Bücker et al. 2013), have lechmanicidal activity (Correa et al. 2006) and antiviral, antibacterial (Smânia et al. 1995, 2003) and anti-inflammatory activity (Kuanget et al. 2010). Pigment characterization, and its biosynthesis have been described by several authors. It is indicated that a phenoxazine-3-one (a nitrogen heterocycle) type structure is responsible for the above activities and for the red color pigment of these strains (Achenbach & Blüm 1991).

Phenoxazine-3-one type structure occurs as the central core of a number of naturally occurring chemical compounds such as the antibiotic actinomycins (polypeptide antibiotics), litmus (7-hydroxyphenoxazone mix), and orcein (7-hydroxyphenoxazone mix). Cinnabarin has a basic ring similar to that of actinomycin D, which is an antibiotic used routinely to treat certain forms of cancer. However, actinomycin D is very toxic to humans. By contrast, cinnabarin at a concentration of 0.31 mg/mL had no effect on mouse neuroblastoma cells, and at doses of 1000 mg/kg it did not cause toxic effects in mice. In addition, at concentrations of 0.31 mg/mL, it produced a 4-fold reduction in the titers of the rabies virus and cinnabarinic acid is active against several Gram-positive bacteria of the *Streptococcus* group (Jiang et al. 2011).

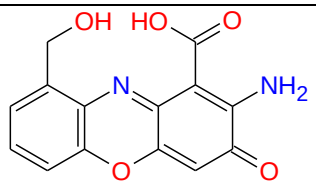
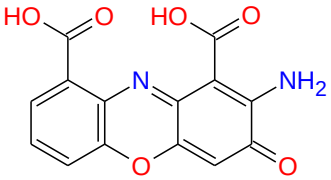
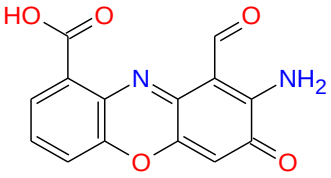
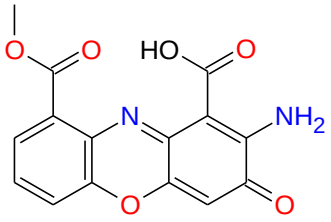
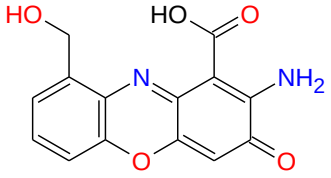
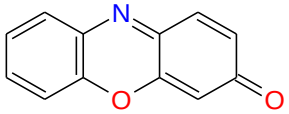
Cinnabarinic acid is a red pigmented phenoxazinone synthesized as a by-product of the kynurenine pathway, which derives from the condensation of two molecules of 3-hydroxyanthranilic acid. Fruiting bodies and mycelial growth of *Pycnoporus cinnabarinus* were screened and found to possess antibacterial properties (Fajana et al. 1999, Shittu et al. 2005). The concentrated culture fluid of *Pycnoporus cinnabarinus* showed biological activity against a variety of bacterial strains, with maximal inhibitory effect for Gram-positive bacteria of the genus *Streptococcus*. Laccase secreted by the fungus oxidizes the precursor 3-hydroxyanthranilic acid to cinnabarinic acid, a reaction that is necessary for the production of antibacterial compounds. The biological activity of the concentrated culture of *Pycnoporus cinnabarinus* was nearly identical with that of cinnabarinic acid, synthesized by purified laccase in vitro (Eggert 1997). In another study, a liquid culture filtrate of *Pycnoporus cinnabarinus* showed positive antibacterial effects against the growth of the Gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa* as well as Gram-positive *Staphylococcus aureus*. The culture filtrate was also used against mycelial growth and mycelial weight of three plant pathogenic fungi, *Botrytis cinerea, Colletotrichum gloeosporioides* [*Glomerella cingulata*] and *Colletotrichum miyabeanus,* showing good inhibitory effects (Imtiaj et al. 2007).

Anti-tumor effects of polysaccharides extracted from the mycelial culture of *Pycnoporus cinnabarinus* and administered intraperitoneally into white mice at a dosage of 300 mg/kg inhibited the growth of Sarcoma 180 and Ehrlich solid cancers by 90%. More over cinnabarinic acid has been shown to possess strong apoptosis-inducing activity and results in the production of reactive oxygen species with loss of mitochondrial membrane potential and reduces caspase activation. Its biological function is largely unknown, however, it is a modulator of immune response in autoinmmune disorders or an immune modulator (Hayashi et al. 2001). Indoleamine-2,3-dioxygenase is induced by proinflammatory cytokines, such as interferon-γ; therefore, the kynurenine pathway is activated in the context of neuroinflammation, as occurs in the ischemic brain or in the brain of patients with HIV infection. Neuroactive metabolites of the kynurenine pathway, such as quinolinic acid, kynurenic acid, and xanthurenic acid, are involved in synaptic signaling and in mechanisms of neurodegeneration/neuroprotection. Quinolinic acid, acts as an orthosteric agonist of N-methyl-d-aspartate (NMDA) receptors, whereas kynurenic acid and its synthetic derivatives are competitive antagonists at the coagonist glycine site of NMDA receptors. Recently, cinnabarinic acid was reported as a novel endogenous orthosteric agonist of mGlu4 receptors endowed with neuroprotective activity (Fazio et al. 2012).

Among the natural compounds of the phenoxazine class are tramesanguin, a crystaline pigment that was isolated from these *Pycnoporus* strains or *Coriolus sanguineus* and questiomycin an extract from certain *Streptomyces* and *Waksmania* species. Besides cinnabarin, cinnabarinic acid and tramesanguin and at least 6 more different aminophenoxazine derivative compounds have been reported in these three *Pycnoporus* strains. These different aminophenoxazine derivatives can be synthesized enzymatically at room temperature and without addition of dangerous chemicals during bioprocesses catalyzed by laccase or phenozaxineone synthase, as well as cinnabarin, cinnabarinic acid and tramesanguin producing interesting color with applications such as textile and hair dyes. Phenoxazine derivatives can be used as platform chemicals to synthesize other compounds using synthetic biotechnology processes based on laccases, tyrosinases, phenoxazine one synthase and other enzymes with broad applications (Forte et al. 2010).

The presence of cinnabarin or poliporin in *Pycnoporus sanguineus* was reported first by Bose (1946) followed by other researchers (Gripenberg 1951, 1958, 1963, Cavill et al. 1953, Achenbach & Blümm 1991). Polystictin or 1-carboxy-2-amino-9 hydroxy methyl phenoxazin-3-one (de Oliveira et al. 2007); tramesanguin or 1-carboxy-2-amino-9 formylphenoxazine-3-one (Gripenberg 1958); cinnabarinic acid (2-Amino-3-oxo-3H-phenoxazine-1,9-dicarboxylic acid); α-aminophenoxazone; pycnoporin or 2-Amino-9-[hydroxy(methoxy)methyl]-3-oxo-3H-phenoxazine-1-carboxylic acid (Gripenberg 1951; Dias & Urban 2009) were also reported. Achenbach & Blümm (1991), reported a 2-amino-phenoxazin-3-one type phenoxazine ether pycnosanguin and an unsubstituted phenoxazin-3-one in *Pycnoporus sanguineus* carpophores. Dias & Urban (2009) added to the list, 2-amino-9 formylphenoxazone-1-carbonic acid; 9-hydroximethyl-2-methylamino phenoxazone-1-carbonic acid methyl ester; and the previous reported un-substituted phenoxazone or 3H-Phenoxazin-3-one; o-acetyl cinnabarin (1-carboxy-2-amino-9 acetyl phenoxazine-3 one). The structures of some of these compounds are shown in Table 4.

Table 4 Phenoxazone compounds in *Pycnoporus* strains.

Common name	IUPAC name	Structure
Cinnabarin	2-amino-9-(hydroxymethyl)-3-oxo-3*H*-phenoxazine-1-carboxylic acid	
Cinnabarinic acid	2-amino-3-oxo-3*H*-phenoxazine-1,9-dicarboxylic acid	
Tramesanguin	2-amino-1-formyl-3-oxo-3*H*-phenoxazine-9-carboxylic acid	
α-Aminophenoxazone	2-amino-9-(methoxycarbonyl)-3-oxo-3*H*-phenoxazine-1-carboxylic acid	
Pycnoporin	2-amino-9-(hydroxymethyl)-3-oxo-3*H*-phenoxazine-1-carboxylic acid	
Phenoxazone	3*H*-phenoxazin-3-one	

Ethnomycological notes mentioned that *Pycnoporus sanguineus* is used to help relieve symptoms of arthritis, gout, styptic, sore throats, ulcers, tooth aches, fevers, and hemorrhages in native people from Australia, Brazil and Africa. Moreover, *Pycnoporus sanguineus* liophilized infusion in potato broth displays antibacterial activity against *Escherichia coli*, *Streptococcus pyogenes* (Group A *Streptococci*), *Pseudomonas aeruginosa*, *Salmonella typhiand* and *Staphylococcus aureus* (Smânia et al. 1995). Among 11 bacteria isolated from food, cinnabarin

inhibited the bacteria growth by 0.0625 mg/mL (Smânia et al. 1995). *Klebsiella pneumonia* was the least sensitive (>4.0 mg/mL). Smânia et al. (1997), reported that *Pycnoporus sanguineus* was grown in potato dextrose broth and the best conditions for producing cinnabarin were initial medium pH 9.0, 25°C, under light. Antimicrobial activity has been reported from *Pycnoporus sanguineus* mycelium grown in petri dishes against *Bacillus subtilis, Burkholderia unamae, Escherichia coli, Listeria monocytogenes, Shigella flexneri, Salmonella typhi, Staphylococcus aureus and Streptococcus agalactiae.* From fruiting bodies, *Pycnoporus sanguineus* cultivated on oak sawdust showed the highest yield of cinnabarin (68 mg/g), and it was active at minimal inhibitory concentrations between 41-140 mM against *Escherichia coli, Listeria monocytogenes, Salmonella typhi*, and *Staphylococcus aureus.* It also mentioned that cinnabarin obtained from mycelium grown in petri dishes with potato-dextrose agar, whole wheat flour agar or either phosphate agar containing 3% w/v wheat grain, oat straw or aquatic lily, were more active than that from fruiting bodies; they reported *Pycnoporus sanguineus* growth, pigment production and antimicrobial activity in liquid and solid-state cultures (Acosta-Urdapilleta et al. 2010).

Uzan et al. (2010) described the oxidation of monomeric and dimeric non-phenolic lignin model compounds such as veratryl alcohol and adlerol by laccase of *Pycnoporus sanguineus*, testing different natural and synthetic redox mediators at different pH. The 1-Hydroxybenzotriazole (HBT), a synthetic mediator, was the most efficient redox mediator and allowed 100% oxidation of veratryl alcohol into veratraldehyde and 86% oxidation of adlerol into adlerone. It has been reported that *Pycnoporus sanguineus* is able to produce fluorescence compounds when grown in liquid or on solid substrates (Acosta-Urdapilleta et al. 2010).

Pycnoporus cinnabarinus (Jacq.) Fr., (1881) is able to produce the three pigments cinnabarin, cinnabarinc acid and tramesanguin (Sullivan & Henry 1971, Eggert et al. 1996). However, it produces cinnabarinic acid as the main metabolite and it is responsible for its antimicrobial activity. Concentrated liquid culture of this strain has a maximal inhibitory effect towards Gram-positive bacteria of the genus *Streptococcus*. Inhibitory antimicrobial activity was higher for Gram-positive than Gram-negative bacteria (Eggert 1997). Cinnabarinic acid production by this strain has been studied by several researchers and their work has granted very interesting applications. Gripenberg (1958) reported cinnabarinic acid is biosynthesized from 3-hydroxyanthranilic acid units formed from tryptophan transformation products. Cinnabarinic acid, can also be obtained in a non-enzymatic process by action of active oxygen species and various transition metals (Eggert et al. 1995). For instance compounds such as o-aminophenol often couple spontaneously to form cyclic phenoxazine-one derivatives by an oxidation process, because of its high reactivity. 3-hydroxyanthranilic acid is coupled spontaneously to a phenoxazin-one derivative, cinnabarinic acid. In addition, it can be obtained enzymatically by peroxidase, catalase, tyrosinase or laccase. However, laccase is not capable of non-phenolic lignin structure degradation without a chemical redox mediator because of their low redox-potentials (Eggert et al. 1995).

To overcome the redox potential barrier, *Pycnoporus cinnabarinus* produces a metabolite that can mediate the oxidation of non-phenolic substrates by laccase; which is 3-hydroxyanthranilic acid, a laccase mediator. O-aminophenol and 3-hydroxyanthranilic acid are metabolites of the kynurenine pathway and act as precursors of cinnabarinic acid. Anthranilic acid is an intermediate of tryptophan synthesis and 3-hydroxyanthranilic acid occurs by hydroxylation of anthranilic acid in the shikimic acid pathway. The reaction resembles the synthesis of actinomycin D by the blue copper enzyme, phenoxazinone synthase, in *Streptomyces antibioticus* and other pigments (Turner 1971). Color phenoxazinone structures substrates can be obtained by laccase activity of *Pycnoporus cinnabarinus* such as 4-methyl-3-hydroxyanthranilic acid (Osiadacz et al. 1999), 3-amino-4-hydroxybenzenesulfonic acid (Forte et al. 2010), and 3-hydroxyorthanilic acid when glucose is the carbon source, then cinnabarinic acid can be produced by laccase induced oxidation of 3-hydroxyanthranilic acid in batch cultures according to Eggert et al. (1995). In *Pycnoporus cinnabarinus*, laccase purified from liquid cultures was found to be important for synthesis of the phenoxazinone pigments which give the fruiting bodies a red color (Eggert et al. 1995).

The phenoxazinone pigments and, laccase activity can also be linked to the antimicrobial activity of this organism (Eggert et al. 1997). A strain of *Pycnoporus cinnabarinus* ss3 was reported to produce up to 29,000 U/L of laccase in the presence of ferulic acid as aromatic inducer. The presence of two genes encoding for two laccase isoenzymes, LAC I and LAC II were reported with applications in enzymatic delignification of wheat straw pulp and for the reticulation of phenolic compounds into agro-polymers (Figueroa-Espinoza & Rouau 1998). The results obtained *in vitro* confirm the role of laccase in the synthesis of this chromophoric compound as a product of the coupling reaction between unstable aminophenolic radicals (Eggert et al. 1995). The effect of ethanol to increase laccase yield by *Pycnoporus cinnabarinus* ss3, compared to that of ferulic acid indicated, in the presence of 35 g/L ethanol, laccase activity (266,600 U/L) and productivity (19,000 U/L day) were nine- and five-fold higher than those of ferulic acid-induced cultures, and 155 and 65 fold higher than those of control cultures, respectively (Herpoël et al. 2002).

A temperature-dependent change of cinnabarinic acid production was investigated using 3-hydroxyanthranilic acid and laccases to produce cinnabarinic acid in stirred tank reactors. The best inducing conditions included liquid media suplemented with 5 g/L maltose and 2.5 g ammonium tartrate at 26ºC for large scale applications (Göçenoğlu & Pazarlioglu 2014). 3-hydroxyanthranilic acid is produced by interferon-gamma-primed mononuclear phagocytes in large amounts, therefore, this reaction is very important in clinical studies, since 3-hydroxyanthranilic acid acts as a powerful scavenger of reactive oxygen species. In mammalian tissues, cinnabarinic acid is produced by the peroxyl radical-mediated oxidation of 3-hydroxyanthranilic acid and it might prevent oxidative damage in mammalian tissues (Eggert et al. 1995).

Transformation of the selected precursors into intensive and non-toxic products in liquid culture by actively growing fungal biomass was studied. Stable lasting products were obtained using oxygen as a clean oxidant. Further applicability of the fungi in the synthesis of new colored molecules, is currently promising. Colored products can be used in applications in cosmetic and food industries and as textile or hair dyes. All these products have valuable dyeing properties (Polak & Jarosz-Wilkołazka 2010). In addition to their excellent coloring properties, phenoxazinones are non-toxic and can be synthesized at room temperature and without addition of dangerous chemicals during bioprocesses catalyzed by laccase. Additionally, the phenoxazinone part of actinomycin-D and its amino derivatives are known to exhibit various fluorescence properties, which have been used in the development of probes in the field of histochemistry and molecular biology

Pycnoporus coccineus (Fr.) Bondart & Singer (1941), is the only *Pycnoporus* strain reported to have phenoxazinone synthase enzyme (Nair & Vining 1964, Le Roes-Hill et al. 2009), and it is also a member of the blue copper oxidase family, then like laccases and tyrosinases, the reaction mechanism mediated by this enzyme is similar to other multicopper oxidases. Interestingly the mammalian protein, ceruloplasmin, as well as laccase, is a member of the blue copper oxidase class of enzymes that catalyzed 3-hydroxyanthranilic acid coupling to cinnabarinic acid (Eggert et al. 1995). There are just four of these enzymes listed in the enzyme databases, these are: from *Bauhinia monandra, Tecoma stans, Pycnoporus coccineus, Streptomyces griseus subsp. griseus* and the phsA from *S. antibioticus*. The phenoxazine-one sinthase enzyme from *Pycnoporus coccineus* requires Mn^{2+} and riboflavin 50-monophosphate and is typically inactive in the absence of flavin mononucleotide (Le Roes-Hill et al. 2009). Its applications are important in several industrial processes such as in the textile industry for dyeing natural fabrics or hair (Shin et al. 2001, Le Roes-Hill et al. 2009). When 3-hydroxyorthanilic acid is converted into aminophenoxazine derivatives with sulfonyl groups, solubility is increased in water and so can be considered as a green chemistry process, it is colorful and can be used as textile dyes for dyeing natural fabrics and for biosensors.

Aroma

An aroma is a chemical compound that has a smell or odor, and when it is sufficiently volatile, it can be transported to the olfactory system. Flavors affect both the sense of taste and

smell, whereas fragrances affect only smell. Flavors tend to be naturally occurring, and fragrances tend to be synthetic. Aroma compounds can be found in food, wine, spices, perfumes, fragrance oils, and essential oils. Indeed, many of the aroma compounds play a significant role in the production of flavorants (Fahlbusch et al. 2003). For years companies had directed their attention towards natural flavor compounds of biological origin. Among them plants had been an important source of essential oils and flavors, however, active components are often present in minor quantities making isolation difficult and the flavor products are expensive.

Most available flavor compounds are produced via chemical synthesis or extraction, however, sometimes there is formation of undesirable racemic mixtures and there is growing aversion of the consumer towards chemicals added to food, cosmetics and other household products. Because of that, a directly viable alternative route for flavor synthesis is based on microbial processes, i.e. fermentation (de novo) and bioconversion of appropriate precursor compounds based on microorganisms (bacteria, fungi, yeasts) and their enzymes.

In parallel a high demand for natural ingredients in food, cosmetics and other industries, has directed researches to aroma compounds with desirable antioxidant properties such as the hydroxyl cinnamic acids, due mainly to its occurrence in nature and radical scavenging activity. These compounds exist predominantly as hydroxybenzoic and hydroxycinnamic acids. Metabolic pathways of p-coumaric acid had been suggested mainly to explain the ability of *Pycnoporus cinnabarinus* to produce aromatic compounds based on the different metabolites identified. *Pycnoporus cinnabarinus* is able to produce methyl anthranilate de novo in culture conditions combining low nitrogen concentration, maltose as carbon source and uncontrolled culture pH (Gross et al. 1990). *Pycnoporus cinnabarinus* has been reported as a vanillin producer from ferulic acid (Gross et al. 1991, Stentelaire et al. 2000).

A new two-step process for the production of pure vanillin from autoclaved maize bran was designed involving *Aspergillus niger* and *Pycnoporus cinnabarinus*. Two strategies were defined using autoclaved maize bran; to improve *Aspergillus niger* grown on sugar beet pulp to produce high levels of polysaccharide-degrading enzymes, including feruloyl esterases, and to transform ferulic acid into vanillic acid (Bonnin et al. 2002). Certain strains of *Pycnoporus cinnaburinus* under laboratory culture conditions can form methyl anthranilate, an important natural flavouring and secondary metabolites important in biotechnology areas of food. There is a hypothetical relationship between aroma and pigment synthesis in *Pycnoporus* strains. Benzaldehyde was obtained using L-phenylalanine as substrate with *Pycnoporus cinnabarinus* (Lomascolo et al. 1999). Fungal transformation of p-coumaric acid into caffeic acid, a strong antioxidant is possible by *Pycnoporus cinnabarinus* cultures grown with high p-coumaric acid feeding rates (Estrada-Alvarado et al. 2003). The white-rot basidiomycetes, and especially the genus *Pycnoporus*, represent major biotechnological agents for generating, de novo or by bioconversion, natural aromas for industry when grown in standard media or in the presence of precursors (Lomascolo et al. 2002).

Pycnoporus as enzyme producer

The photomass is the plant matter produced by photosynthesis and lignocellulose is the major component representing around half of this matter. It is composed of polysaccharides (cellulose and hemicellulose) mainly and lignin, representing the most abundant renewable organic resource in soil. Its components are strongly intermeshed and chemically bonded by non-covalent forces and by covalent cross-linkages (Pérez et al. 2002). Polysaccharides are polymers of monosaccharides connected to each other by glycosidic bonds. Among the main functions of these compounds, have been reported as structural components, storage materials and protective substances. Structural polysaccharides are either fibrous polysaccharides, mainly cellulose in higher plants and some algae or chitin in yeast and fungi or matrix polysaccharides, for example arabinoxylans, galactomannans or pectins in plants. Starch, glycogen, some β-glucans, fructans and some galactomannans are examples of storage polysaccharides. Protective polysaccharides include extracellular polysaccharides from microorganisms or exudate gums from plants (Aspinall 1982).

Based on the complex composition of lignocellulose, its degradation is very difficult, this process can be carried out mainly by microorganisms with complex enzymes including hydrolases and phenoloxidases. Some fungi organisms are very efficient degraders of lignocellulosic material. There are two main groups of wood decay fungi, including those in the division Basidiomycota, commonly called Basidiomycetes, and those included in the division Ascomiceta, or Ascomycetes. These fungi are classified according to the way they act on wood. White-rot fungi break down all major wood components (cellulose, hemicelluloses and lignin) and commonly cause bleached rotted wood. Brown-rot fungi primarily decay cellulose and hemicellulose in wood, leaving a brown residue of lignin, and the soft-rot fungi preferentially degrade cellulose (Leonowicz et al. 1999, Rabinovich et al. 2004).

White-rot fungi are capable of degrading some xenobiotic compounds and different kinds of environmental pollutants due to its ligninolytic enzyme system. Ligninolytic enzymes have potential applications in a large number of fields, including chemical, fuel, food, agricultural, paper, textile, cosmetic industrial sectors and more. Species of *Pycnoporus* genus, produce diverse enzymes. There are reports about the production of β-N-acetylhexosaminidase, chitinase, N-acetyl-β-glucosaminidase, α- and β-galactosidases, α-amylase and β-glucosidase, β- fructofuranosidase (or invertase) by *Pycnoporus cinnabarinus* and in *Pycnoporus sanguineus* cellulolytic and hemicellulolytic activities, as well as, xylanases, exo-polygalacturonase, PGase I, α-D-mannosidase, Avicelase, β-glucosidase and oxidases mainly laccases were reported (Lomascolo et al. 2011). Eugenio et al. (2009) reported the strong influence of four carbon sources (maltose, glucose, fructose and sucrose) and four nitrogen sources (ammonium tartrate, sodium nitrate, asparagine and yeast extract) on the activity of laccase from *Pycnoporus sanguineus*.

A mix of sucrose and asparagineinduced the best results (320 mU/mL), however, with an increase of 5 times the asparagine concentration, laccase activity was increased to 820 mU/mL. *Pycnoporus sanguineus* produced laccases using a medium containing 200 mg/L of 2,5-xylidine or 50 g/L of ethanol, the maximum activity was 2019 U/L and 1035 U/L, respectively. The biomass and laccase activity did not show correlation in any case. Ethanol is a cheaper inducer with little toxic effect for laccase production by *Pycnoporus sanguineus* (Valeriano et al. 2009). Three anthraquinonic dyes were used to understand their *in vivo* decolourisation for laccases of *Pycnoporus* strains. *Pycnoporus sanguineus* MUCL 41582 (PS7) and *Pycnoporus cinnabarinus* MUCL39533 (PC330) were used. The first, reported as a producer of laccase and the second, described as laccase-deficient. In medium without dye, laccase activity was observed only by the PS7 strain, however, in the presence of either, Acid Blue 62 (ABu62), Acid Blue 281 (ABu281) and Reactive Blue 19 (RBu19), the laccase activity was also observed with PC330 strain (Vanhulle et al. 2007). *Pycnoporus sanguineus* is a fungus reported as a biosorbent of metal ions. This process depends on pH, initial metal concentration, temperature and biomass loading. This fungus can be reused multiple times and can be used on an industrial scale (Yahaya & Don 2014). *Pycnoporus sanguineus* MUCL 51321 isolated in Gabon was grown in solid and liquid media and showed laccase and manganese peroxidase activities. On the other hand, this fungus was able to discolor at 81% and 97% the orange G (0.05 g/L) and reactive blue 4 (0.3 g/L), respectively (Christiane et al. 2013). *Pycnoporus sanguineus* and *Trametes membranacea* were evaluated for decolorization of dyes Orange II and Black V. Malt and King media supplemented with 0.05 % dyes, either Orange II and Black V at pH 4.5 and 5.0 in the presence or absence of agitation and/or luminosity were used. The decolorization was over 50% for the dyes in all flasks under agitation (Da Paz et al. 2014).

Ganoderma applanatum and *Pycnoporus sanguineus* from Misiones (Argentina) were grown in dyes and kraft black liquor. Black liquor is a kraft pulping process effluent containing solubilized lignin, the primary organic by product from the chemical digestion of lignocellulosic raw materials. Dye decolorization (bromophenol blue, malachite green) were made in solid culture and black liquor degrading studies were conducted in solid and liquid cultures. These experiments validated the dye and lignin degrading ability of both fungi in solid and liquid cultures (Shimizu et al. 2009). Singh et al. (2012) reported the biodegradation of oil palm biomass and its study for

pretreatment of laccase activity by *Pycnoporus sanguineus*. Laccase was produced at room temperature in the presence of Kirk medium supplemented with glucose, ammonium nitrate and corn steep liquor. Laccases resisted temperatures from 10 to 70°C and pH of 2.5-6. It was observed that the selective degradation of lignin was 4 weeks. Weight and component losses of oil palm trunk chips after decay were evaluated and analyzed by scanning electron microscopy.

The maximum laccase activity was 1.007 U/L after 10 days of incubation. *Pycnoporus sanguineus* was grown in bothshake flasks and stirred tank reactors, and Ag nanoparticles were synthesized. The morphology, structure uniformity and concentration of Ag nanoparticles were evaluated by UV-vis spectroscopy, dynamic light scattering, atomic absorption spectroscopy and transmission electron microscopy. Proteins with molecular weight of 37.5–69.6 kDa (SDS-PAGE) were related with biosynthesis of Ag nanoparticles. The yield of Ag nanoparticles in the stirred tank reactor was higher with controllable sizes than those produced in shake flasks studies (San et al. 2013).

Omic technologies and strategies on *pycnoporus* genus

Recently developed technologies in genetics and informatics gave the scientific community the tools to study from a holistic point of view, the complexity of biological processes that take place in a cell, tissue or organism as well as the molecules involved in them. These new disciplines can be summarized by the term *"Omic"* (Horgan & Kenny 2011). This term first emerged in the field of genomics, and then new disciplines as fields of proteomics, metabolomics, and transcriptomics adopted it. The objective of the omic technologies is the detection of genes (genomics), mRNA (transcriptomics), proteins (proteomics) and metabolites (metabolomics) in a biological sample (Kell 2007). The integration of all these disciplines is now called systems biology (Kitano 2002, Breitling 2010). The basic aspect of these approaches is that a complex system can be understood more thoroughly if considered as a whole, the posterior analysis of the information generated by these strategies could help in the formulation of different hypothesis, contrasting with traditional strategies in which a hypothesis is generated prior to conducting the experiments (Kell & Oliver 2004).

The omic strategies have many applications and potential. They can be applied not only for the better understanding of physiological processes that take place in a biological sample; they are also useful to understand disease processes (Horgan & Kenny 2011). These techniques, integrated, could also help to discover new therapeutics and the targets for its action (Ulrich-Merzenich et al. 2007, Ahn & Wang 2008, Sleno & Emili 2008, Caberlotto & Lauria 2015), along with enzymes and other proteins or metabolites of clinical or industrial interest from different sources (Fridman & Pichersky 2005, Rochfort 2005, Tang & Zhao 2009, O'Flaherty & Klaenhammer 2011).

The omic technologies have been applied to the study of different fungi, to study their pathogenicity (Yoder & Turgeon 2001; Van de Wouw & Howlett 2011) or for their medical and industrial potential (Keller et al. 2005, Carberry & Doyle 2007, Gonzalez-Fernandez & Jorrin-Novo 2011, Grigoriev et al. 2011). One of the main interests for the application of the omic technologies in fungi, is the identification of powerful enzymes with the potential to breakdown plant biomass for applications in biorefinery (Polizeli et al. 2005, Maciel & Ribeiro 2010, Kubicek 2012, Ferreira et al. 2013), degrading of pollutants in environmental applications (Wesenberg et al. 2003, Singh & Singh 2014), or for their use in the food, pharmaceutical and cosmetic industries (Hyde et al. 2010, Tilay et al. 2010, Lopes et al. 2013).

Genomic of Pycnoporus genus

Among the most important motivations to sequence fungal genomes is to highlight their impact over human well-being especially in medicine, health care, biotechnology, food security, alternative energy, and maintaining the ecological integrity (Robbertse & Tatusova 2011). The first fungal genome was from *Sacharomyces cerevisiae* published in 1996 (Goffeau et al. 1996). From this report, many sequencing initiatives to obtain the genomic information from the most representative fungal groups were established, generating the report of many fungal genomes

(Grigoriev et al. 2014). The analysis of all this information could help to discover new molecules with biotechnological potential.

The first genome from a *Pycnoporus* genus species was recently published (Levasseur et al. 2014). The white-rot fungus, *Pycnoporus cinnabarinus* is a model for the study of plant cell wall decomposition and is used for a number of applications in green and white biotechnology. The 33.67 Mb genome of *Pycnoporus cinnabarinus* BRFM137 was sequenced and assembled, with a G+C content of 52.6% and the presence of 10,442 predicted genes (ORFs). Of this number, 5,417 were functionally annotated using a phylogenomic procedure and grouped in three categories; biological processes, cellular components and molecular function. Among the annotated genes, several enzymes involved in carbohydrate metabolism, lignin degrading and wood decay were identified (Levasseur et al. 2014). The sequencing of the genome of other *Pycnoporus* genus species as *Pycnoporus coccineus* strains CIRM1662 and BRFM310, and *Pycnoporus sanguineus* BRFM1254 are in process, according to the information generated by the "Survey of lignocelloulolytic capabilities over the order Polyporales (Fungi, Basidiomyces)" project deposited in the Joint Genome Institute portal (JGI, 2015) and in the publication of Busk et al. (2014).

Trascriptomic of Pycnoporus genus

Transcriptomics is the global analysis of gene expression at the RNA level. This technique is important to understand the gene functions and their abundance for a specific developmental stage or physiological condition in the organism under study (Wang et al. 2009). At present transcriptomics is a robust, high-throughput, cost-effective technology capable of simultaneously quantifying tens of thousands of defined mRNA species in a miniaturized, automated format (Hegde et al. 2003). Recently many transcriptomic studies have been performed on fungi. Examples are yeasts such as *Candida albicans* and *C. dubliniensis* (Bruno et al. 2010, Grumaz et al. 2013), filamentous fungi such as *Aspergillus fumigatus* (Gautam et al. 2008), *A. nidulans* (Sibthorp et al. 2013), *Colletotrichum orbiculare* (Gan et al. 2012) and *Trichoderma* spp. (Seidl et al. 2009, Atanasoba et al. 2013), as well basidiomycete fungi such as *Coprinopsis cinerea* (Cheng et al. 2013), *Phanerochaete carnosa* (MacDonald et al. 2011), *Phanerochaete chrysosporium* (Wymelenberg et al. 2009), and *Pleurotus ostreatus* (Castanera et al. 2012), among others.

The first trancriptomic study on a *Pycnoporus* genus member was performed by Rohr et al. (2013). In their study the fungus *Pycnoporus sanguineus* strain BAFC2126 was grown in media supplemented with copper sulfate to favor the transcription of laccases, glyoxal oxidases and manganese peroxidases prior to the trascriptomic analysis. Results of the study show 7,303 transcripts were obtained, of this number 4,732 were assigned by similarity, and identified as 178 potential enzymes with activity over carbohydrates as glycoside hydrolases, glycosyltransferases, carbohydrate esterases and polysaccharide lyases, as well enzymes related to lignocellulose degradation as laccases, multicopper oxidases, lignin peroxidases, manganese peroxidases, and versatile peroxidases, among others. Terpenoid biosynthesis enzymes were also identified. This study has established that *Pycnoporus sanguineus* has great potential for the production of enzymes that may be applied in many industries. The transcriptomic study of *Pycnoporus coccineus* strains BRFM310 and CIRM1662 are in process (JGI 2105).

Proteomic of Pycnoporus genus

Proteomics is the systematic analysis of the proteins expressed in a cell, tissue or whole organism. This omic technology allows the identification and quantification of large number of proteins related to cellular metabolism, changes in the expressed protein patterns during the growth and development of an individual or in response to environmental factors (Chen & Harmon 2006). Proteomic studies have been performed to identify the proteins presents in the secretions of different fungi, mainly filamentous fungus (Bhadauria et al. 2007). The first fungi proteomic studies were performed by Grinyer et al. (2004, 2005) in *Trichoderma atroviride* and *Trichoderma harzianum* with the aim to understand the mechanism related to the control of pathogenic fungi by these species. Since then, other proteomic studies related to filamentous fungi have been published

(Kim et al. 2007, van Passel et al. 2013). Proteomic studies in basidiomycete fungi have been performed first in the model fungi *Coprinopsis cinérea* (Hoegger et al. 2007) and *Phanerochaete chrysosporium* (Abbas et al. 2005, Sato et al. 2007).

Further studies in *Phanerochaete chrysosporium* to determine the enzymes involved in metabolism of benzoic acid (Matsuzaki et al. 2008), *Trametes hirsuta* grown in different substrates to study the production of laccases (Vasina et al. 2013), *Trametes versicolor* to evaluate the production of pollutant degrading enzymes (Lebrum et al. 2011) were performed. Antihypertensive peptide production by edible mushrooms such as *Agaricus bisporus* and *Pleurotus cystidiosus* have been studied by proteomics (Lau et al. 2012). Secretome studies in *Pycnoporus* genus species are limited. Only the secretome of *Pycnoporus cinnabarinus* strain BRFM137 was analyzed in the work of Lavassier et al. (2014). The fungus was grown in different conditions in liquid phase with the presence of combinations of maltose, birchwood, maize bran, and Avicel, as well as, in solid-state fermentation. Findings indicate that in the cultures with maltose-maize and bran-Avicel, in solid-state fermentation, 52% and 55% of the proteins in the secretome were carbohydrases, respectively, while in the maltose and birchwood media only 41 and 47% of the proteins produced were carbohydrases, respectively.

The production of hemicellulolytic and ligninolytic proteins detected in secretomes, showed different distribution patterns depending on growth conditions. Even if the genome of the fungus is available, the identity and function of many of the secreted proteins remains to be elucidated. The proteomic study of the secretome of fungi may lead to the discovery of novelty enzymes and help in the understanding of the strategies and synergistic effects of fungal extracellular biocatalysts (Bouws et al. 2008).

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