Biotechnological, nutritional and therapeutic uses of *Pleurotus* spp. (Oyster mushroom) related with its chemical composition: A review on the past decade findings

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Abstract

Background: The particular characteristics of growth and development of mushrooms in nature result in the accumulation of a variety of secondary metabolites, several of them with biological activities. The genus *Pleurotus* is a cosmopolitan group of mushrooms with high nutritional value and therapeutic properties, besides a wide array of biotechnological and environmental applications.

Scope and approach: The present report aims to provide a critical review on aspects related to chemical compounds isolated from the genus *Pleurotus* with possible biotechnological, nutritional and therapeutic uses. Investigations on the genus have immensely accelerated during the last ten years, so that only reports published after 2005 have been considered.

Key findings and conclusions: The most important *Pleurotus* species cultivated in large scale are *P. ostreatus* and *P. pulmonarius*. However, more than 200 species have already been investigated to various degrees. Both basidiomata and mycelia of *Pleurotus* are a great renewable and easily accessible source of functional foods/nutraceuticals and pharmaceuticals with antioxidant, antimicrobial, anti-inflammatory, antitumor and immunomodulatory effects. A series of compounds have already been precisely defined including several polysaccharides, phenolics, terpenes and sterols. However, intensification of structure determination is highly desirable and demands considerable efforts. Further studies including clinical trials need to be carried out to ascertain the safety of these compounds as adequate alternatives to conventional drugs. Not less important is to extend the search for novel bioactives to less explored *Pleurotus* species.

Keywords: β-glucan, functional foods, mushrooms, natural products, submerged cultures.
1. Introduction

Mushrooms have been regarded as gourmet cuisine across the globe since antiquity for their unique taste and subtle flavor. They are considered as sources of important nutrients including dietary fiber, minerals, and vitamins, in particular, vitamin D (He, Perera & Hemar, 2012). More than 2,000 species of mushrooms exist in nature, but only around 25 are widely accepted as food and few are commercially cultivated (Valverde et al., 2015). Recently, they have become increasingly attractive as functional foods due to their potential beneficial effects on human health. Hence, food industry is especially interested in both cultivated and wild edible mushrooms. The most extensively cultivated mushroom worldwide is *Agaricus bisporus* (J. E. Lange) Emil J. Imbach., followed by *Lentinula edodes* (Berk.) Pegler and *Pleurotus ostreatus* (Jacq. ex Fr.) P. Kumm. Mushrooms production is continuously increasing. The commercial production in 2012 hit 7,959,979 tonnes of mushrooms, with China accounting for most of the production (5,150,000 tonnes), while 1,869,091 tonnes were harvested in Europe (Grujic et al., 2015). Due to the increase in population and consumption, the world demand for mushrooms is projected to grow 15% a year (Kamarudzaman et al., 2015).

The genus *Pleurotus* (Fries) Kummer (Basidiomycota, Agaricales) was defined by Paul Kummer in 1871. It is a cosmopolitan group of mushrooms with high nutritional value and therapeutic properties, besides a wide array of biotechnological and environmental applications (Knop, Yarden & Hadar, 2015). Usually regarded as oyster mushrooms, these edible basidiomycetes are among the most popular worldwide, as much as they achieved the third position in the production of edible mushrooms, behind the species of the genus *Agaricus* and *Lentinula* (Fernandes et al., 2015). The most important *Pleurotus* species cultivated in large scale are *P. ostreatus* and *P. pulmonarius* (Fr.) Quél. (Bazanella et al., 2013). *P. pulmonarius* has been often marketed by spawn
manufacturers and cultivators under the incorrect name "Pleurotus sajor-caju". The real Pleurotus sajor-caju (Fr.) Singer is in fact a separate species of mushroom, which was returned to the genus Lentinus by Pegler (1975), and is correctly named Lentinus sajor-caju (Fr.) Fries (Buchanan, 1993).

Since the first report of hypotensive activity of the Pleurotus mushroom in a mouse model in 1986, many researchers have demonstrated their medicinal potentialities and classified them as 'mushroom nutraceuticals'; that were posteriorly added to the group of functional foods (Patel, Narain & Singh, 2012). In the last decade, the number of patents and scientific articles regarding the genus Pleurotus has exponentially increased, with an increment of more than 2-fold in the total of scientific research/review articles in the last 5 years (Figure 1).

Extensive research on cultivation techniques (Gregori, Svagelj & Pohleven, 2007; Carvalho, Sales-Campos & Andrade, 2010), chemical composition and nutritional profile (Reis et al., 2012; Atri et al., 2013; Maftoun et al., 2015) has been done in the last ten years, along with a comprehensive account of the biotechnological capabilities of the genus Pleurotus including enzyme production (Inácio et al., 2015a; Knop, Yarden & Hadar, 2015) (Figure 2). More recently, the scientific reports referring to Pleurotus species have also focused on novel approaches for taxonomic issues (Menolli Jr., Breternitz & Capelari, 2014; Maftoun et al. 2015), isolation and characterization of new functional compounds, besides the in depth-study of their medicinal properties (Khan & Tania, 2012; Patel, Narain & Singh, 2012; Yahaya, Rahman & Abdulhah, 2014).

In view of the above, this review aims to summarize and evaluate the past decade findings related to biotechnological, nutritional and therapeutic uses of Pleurotus sp. with special attention to novelties regarding their chemical composition. This includes
discussion of the main isolated and identified compounds or fractions and their corresponding bioactivities.

2. Biodiversity and Taxonomy

As of 2015 the Index Fungorum lists 202 species in the *Pleurotus* genus. Table 1 presents the most studied species in the past ten years, the main areas of publications regarding these mushrooms, as well as their geographical distribution worldwide.

Species delimitation within the *Pleurotus* genus has been a complex issue for decades (Menolli Jr., Breternitz & Capelari, 2014). Years ago, Kitamoto et al. (2004) pointed out the main causes of the taxonomic controversy involving *Pleurotus* species: initial misidentification, absence of type specimens, instability of morphological characters due to environmental changes, limited reports on physiological characteristics, and the lack of mating compatibility studies. Fortunately, in recent years the adoption of biochemical and molecular approaches has brought some clarifications for species delimitation in the genus, mainly when combined with morphology and sexual compatibility (Menolli Jr., Breternitz & Capelari, 2014). The currently adopted methodologies of identification include isozyme electrophoresis, sequence analysis of ribosomal DNA, internal transcribed spacer region (ITS), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), restriction fragment length polymorphism (RFLP) and mating compatibility testing (Maftoun et al., 2015). Recently, molecular approaches made it possible to confirm the taxonomic status of some important *Pleurotus* varieties such as *P. eryngii*, *P. ferulae*, and *P. elaeoselini*. It also enabled reclassifications and the identification of new species (Zervakis et al., 2014).
In 2009, sequencing of the *P. ostreatus* genome was completed. Thanks to this accomplishment, a broad picture of the ligninolytic peroxidase gene family has been obtained. Besides, molecular techniques have also enabled progresses such as targeted gene replacement, RNAi-based gene silencing, and overexpression of genes in *P. ostreatus*. By this way, the recent information of the genomics of *P. ostreatus* secondary metabolism will allow an upgrade in the production of these compounds (Knop, Yarden & Hada, 2015).

As a more affordable option to expensive molecular techniques, the diffuse reflectance infrared Fourier transform (DRIFT) has also been used for studying the molecular composition and for identifying biological samples. It consists in a fast, reagent-free, noninvasive and highly specific approach (Movasaghi et al., 2008). Zervakis et al. (2012) used the DRIFT spectroscopy to exam 16 taxa of the genus *Pleurotus*, concluding that it was a fast, reliable, and cost-efficient methodology for the classification of pure cultures from closely related mushroom species.

### 3. Nutritional Aspects

*Pleurotus* spp are famous for owning all three properties expected from a food — nutrition, taste, and physiological functions — being thus appreciated for both their sensory characteristics and outstanding nutritional profile. The terpenes, lactones, amino acids, and carbohydrates of their composition determine a range of precious aromas and flavor characteristics to their fruiting body and mycelial biomass (Smiderle et al., 2012). *P. ostreatus*, the most popular species of the genus, is commonly used in the preparation of soups, in stir-fry recipes with soy sauce or eaten stuffed. *P. eryngii* (DC.) Quél., another species with gastronomic prestige, is considered ideal for vegetarian dishes.
(consumed fresh), being served sautéed, grilled, braised, stewed, or boiled (Reis et al., 2012).

Concerning the amount of crude protein, mushrooms are ranked below animal meats, but well above most other foods, including milk, which is an animal product. Not to mention the fact that mushroom proteins contain all nine essential amino acids required by humans, enabling their use as a substitute for meat diet (Kakon, Karim & Sah, 2012). However, their nutritional supremacy in relation to the vegetarian diet is also virtue of their chitin rich cell wall that acts as a source of dietary fiber, along with their vitamin content (including thiamine, riboflavin, ascorbic acid, ergosterine, and niacin), considerable contents of micro and macro-elements as phosphorus and iron, carbohydrates and very low fat tenor (Maftoun et al., 2015).

Fresh fruiting bodies of Pleurotus spp contain 85–90% moisture (Khan & Tania, 2012), and the moisture percentage depends on the mushroom species besides other parameters related to harvest, growth, culinary and storage conditions (Reis et al., 2012). Atri et al. (2012) investigated the nutritional composition of P. floridanus Singer, P. pulmonarius, P. sapidus Quél., P. cystidiosus O. K. Mill. and P. sajor-caju (Fr.) Sing and reported, on dry weight basis, contents of carbohydrates of 85.86–88.38%, proteins 0.98–2.17%, crude fat 0.62–0.84%, crude fibers 2.76–3.12% and ash 1.03–2.20%. In turn, Khan & Tania (2012) found some diverse values in their review study on the nutritional value of P. ostreatus, P. sajor-caju, P. florida (Mont.) Singer, P. cystidiosus, P. geesteranus Singer, P. eryngii, P. tuber-regium (Fr) singer and P. flabellatus (Berk. & Br.) Sacco. They found, on dry weight basis, contents of carbohydrates ranging from 36 to 60%, proteins from 11 to 42% and lipids from 0.2 to 8%.
According to Khan & Tania (2012) the carbohydrates in Pleurotus spp. are mainly in the form of polysaccharides or glycoproteins. The most abundant polysaccharides are chitin, \( \alpha \) - and \( \beta \) -glucans, and other hemicelluloses (e.g., mannans, xylans, and galactans). The glucans present various types of glycosidic linkages, such as branched \((1\rightarrow3)\), \((1\rightarrow6)\)-\(\beta\)-glucans and linear \((1\rightarrow3)\)-\(\alpha\)-glucans. The contents of these polysaccharides in the fruiting bodies range from 36 to 60 g/100 g dry weight. Total dietary fiber (mainly chitin) in Pleurotus mushrooms ranges from 10 to 31 g per 100 g dry weight, glucans being also components of soluble or insoluble dietary fibers.

Reis et al. (2012), in an inter-species comparative study on the most widely cultivated and appreciated mushrooms, found that \( P. \) ostreatus and \( P. \) eryngii had higher levels of monounsaturated fatty acids compared to \( Agaricus \) bisporus, \( Lentinula \) edodes and \( Flammulina \) velutipes (Curtis) Singer. Atri et al. (2013) reported that, among the fatty acids, the monounsaturated are present in a higher proportion (37.17–68.29\%) than the saturated ones (26.07–47.77\%) in Pleurotus spp. Maftoun et al. (2015), in their broad compilation data of the nutritional composition of Pleurotus mushrooms, reported that oleic acid (C18:1) was the major monounsaturated fatty acid while linoleic acid (C18:2n–6c) was the major polyunsaturated fatty acid in \( P. \) ostreatus. They also found that the most common monounsaturated fatty acid present in \( P. \) sajor caju, \( P. \) cystidiosus, \( P. \) pulmonarius, \( P. \) floridanus and \( P. \) sapidus was oleic acid. Among the saturated fatty acids (20.2\%), the main contributers were palmitic acid (C16:0; 11.2\%), followed by pentadecanoic acid (C15:0; 2.55\%) and stearic acid (C18:0; 2.53\%). Among the polyunsaturated fatty acids (69.1\%), linoleic acid (68.1\%) was the most common and abundant.

Atri et al (2012) detected three main sugars including sucrose (0.338–2.011 \%), glucose (0.553–0.791\%) and xylose (0.01\%) when analyzing Pleurotus spp. They also found
ascorbic acid content ranging from 0.46 to 0.49 mg/100 g, total phenolics ranging from 6.76 to 16.92 mg of gallic acid equivalents/100 g, β-carotene ranging from 0.134 to 0.221 µg/100 g and lycopene from 0.055 to 0.075 µg/100 g.

For detailed information about essential amino acids, fatty acids, minerals, vitamins, soluble sugars and volatile compounds profiles of the most studied Pleurotus species, the recent review of Maftoun et al. (2015) might be consulted.


4.1. Mushroom Production

The production of mushrooms with better flavor, appearance, texture, nutritional qualities, and medicinal properties at a sustainable cost constitutes a challenge for both industry and independent farmers, since many important operations are involved in this biotechnological process (Sanchéz, 2004). Table 2 summarizes the main cultivation techniques, postharvest treatments and industrial applications of Pleurotus spp. during the last decade.

Numerous articles have reported the viability of producing the Pleurotus spp. basidiome using a wide range of byproducts as substrates, e.g., elephant grass, coast-cross, cotton waste textile, rice straw, by-products of corn production, sawdust, husk of coffee, wheat straw, crushed sugarcane and stalks (banana tree, pea, peanut). Further, several kinds of materials were applied as supplementation, with high biological efficiency being obtained with wheat bran and rice bran (Carvalho, Sales-Campos & Andrade, 2010). However, in the past years, a wide range of alternative, sustainable and green substrates
were used for *Pleurotus* mushrooms production, such as casing material in a compost mixture (Mishra et al. 2013), handmade paper and cardboard industrial wastes (Kulshreshtha et al. 2013), agro-residues combined with biogas digester residues (Chanaky, Malayil & Vijayalakshmi, 2015) and blank/printed paper (Fernandes et al., 2015).

Several studies on the role of the culture medium on mushrooms growth yield and nutritional quality have been done, but Ryu et al. (2014), in an innovative study, have investigated media combinations and components responsible for producing fruiting bodies with a long shelf life. They developed a cultivation medium for extending the shelf life and improving yield of *P. eryngii* mushrooms, increasing the viability of the export procedures. This medium contained 4.5% of crude protein and 15% of nitrogen free extracts.

The cultivation of *Pleurotus* spp. at high temperatures has been studied for a number of mushroom producers and scientists. Considering that some *Pleurotus* species are unable to develop mushrooms at temperatures of more than 28°C, the most important step in the cultivation of these mushrooms under high-temperature conditions (usual condition in tropical countries) is the cold stimulation of the mature mycelium (Chen, 2007). Yingyue et al. (2014) evaluated the effect of cold in the production of *P. pulmonarius* mushrooms. They found that time and the interaction of temperature *versus* time of the cold stimulation treatment were the two major factors influencing density of pinheads, yield per bag and number of mushrooms per bag. Meanwhile, temperature was the major factor influencing the yield per bag and stability. The best performance was recorded following a 12 h cold stimulation at 5°C, suggesting that an appropriate cold
stimulation may enhance the performance of the primordial initiation and yield of *P. pulmonarius* cultivation during the summer season.

*Dulay, Ray & Hou (2015)* investigated the optimal liquid culture conditions for producing *P. cystidiosus* with reference to the nutritional and physical growth factors, as well as with respect to lipid composition. They reported Sabouraud dextrose broth (SDB) as the most suitable culture medium, with maximum mycelial biomass favorably produced in SDB at pH 7.6 when incubated at 28°C. Agitation did not improve mycelial growth of mushrooms.

In the production of *Pleurotus* mushrooms, every ton of mushroom produced generates about five tons of dry spent residual material. This spent mushroom substrate (SMS) has been under-exploited in the past decades, sometimes being used for land filling and crop production only. However, as the correct disposal of SMS is one of the main environmental issues for the mushroom industry, new alternatives for the biotechnological application of this abundant by-product have been explored. Newly, the *Pleurotus* SMS was identified as a low-cost biosorbent for heavy metals removal, and as an effective degradation agent of organochlorine pesticides (*Juárez et al., 2011; Kamarudzaman et al., 2015*).

4.2. Submerged cultivation

Ten years ago about 80% to 85% of all edible-medicinal mushroom products were derived from the fruiting bodies and only 15% proceeded from mycelia extracts (*Lindequist, Niedermeyer & Julich, 2005*). However, the process of producing fruiting bodies or basidiomata is effortful and time-consuming, as it demands large volumes of substrate, space, and qualified labor, factors that hinder research in the laboratory.
Cultivations that are performed in the vegetative phase are much more interesting and functional for research considering that they can be kept in the laboratory, performed on a small and medium scale, and important parameters such as temperature, humidity, pH and aeration can be easily controlled (Inácio et al, 2015a). Thus, submerged cultivation is a promising and still under-explored alternative for the extraction of bioactive molecules in short time, which also allows the mycelia storage for a long period without genetic alterations, benefiting the conservation of biodiversity (Zilly et al., 2011). However, for using the mycelial biomasses, it is necessary to prove that they are similar to fruiting bodies (Soares et al., 2013). Submerged fermentation is also proper for enzyme production and waste bioconversion. With respect to submerged liquid fermentation with Pleurotus spp., recent studies reported the use of potato dextrose broth, amino acids, liquor maiz, reducing sugars (mainly glucose and xylose), casein hydrolyzate, soybean cake, yeast extract and peptone as the main carbon and nitrogen sources. The culture conditions reported refer to temperatures of 25-30 °C and culture pH of 4-6, in addition to the use of static culture or agitation ranging from 100 to 160 rpm (Arango & Nieto, 2013). Most recent publications aimed substrate optimization for maximal production of hydrolytic and oxidative ligninolytic extracellular enzymes.

As members of the white-rot fungi (WRF), Pleurotus spp. present the ability to grow on a variety of lignocellulosic biomass substrates and degrade both natural and anthropogenic aromatic compounds. This occurs by virtue of the presence of non-specific oxidative enzymatic systems, which consist mainly in laccases, manganese peroxidases (MnPs) and versatile peroxidases (VPs) (Hofrichter et al., 2010), besides the newly explored dye decolorizing peroxidases (DyPs) and heme-thiolate peroxidases (HTPs). A lot of information has been accumulated in the past decade concerning the
biochemistry, structure and function of the *Pleurotus* ligninolytic peroxidases (Knop, Yarden & Hadar, 2015).

Recently, the possibility of extending the liquid culture technology for the production of mycelia to the mushroom spawn industry has been studied. Generally the edible mushroom cultivation industry utilizes grain spawn for this purpose. However, it is already known that preparation of grain spawn requires a longer growth period and poses higher risk of contamination compared to liquid spawn (Confortin et al., 2008). Abdulla et al. (2013) investigated the alternative of producing liquid spawn of *P. pulmonarius* by submerged fermentation in a 2-L stirred-tank bioreactor under controlled conditions and assessed its ability to colonise rubber wood sawdust substrate for sporophore production. The ideal liquid spawn cultivation medium contained 20 g L\(^{-1}\) of brown sugar, 4 g L\(^{-1}\) of rice bran, 4 g L\(^{-1}\) of malt extract, and 4 g L\(^{-1}\) of yeast extract (BRMY) with an initial pH of 5.5 and was incubated at 28 °C with agitation speed of 250 rpm and oxygen partial pressure of 30–40%. The maximal dry biomass production of 11.72 ± 5.26 g L\(^{-1}\) was observed after 3 days of fermentation. The authors concluded that liquid spawn has the ability to colonise sterile rubber wood-sawdust as fruiting substrates in a shortened time and to produce a higher yield of sporophores in comparison with the regularly used grain spawn.

4.3. Post Harvested Treatment

The commercial value of mushrooms falls due to quality loss during postharvest storage because the storage conditions are quite different from the growing conditions. This provokes changes in the physiological and molecular mechanisms that lead to deterioration (Li et al., 2013). In the past years, diverse post-harvest treatments have
been investigated in an effort to discover new alternatives for extending the mushroom shelf life: cold storage (Dama et al., 2010), modified atmosphere packaging (MAP) (Guillaume et al., 2010), gamma and electron beam irradiation (Xiong et al., 2009; Fernandes et al., 2012), and coating (Jiang, Feng, & Li, 2012) treatments.

Li et al. (2013) investigated the high carbon dioxide and low oxygen treatment on the sensory characteristics, MDA (malondialdehyde) content, $O_2^-$ production rate, and enzyme activities of SOD (superoxide dismutase), POD (peroxidase), CAT (catalase), and CCO (cytochrome C oxidase) in *P. eryngii*. They reported that 2% $O_2 + 30% CO_2$ treatment could maintain sensory characteristics of the mushroom and significantly prolong its shelf life.

In turn, Zhang et al. (2015) investigated the activity and molecular mechanisms of serine proteinase (Spr) during storage of *P. eryngii*. The activity of Spr in 2% $O_2 + 30% CO_2$-treated mushrooms was notably lower than in the controls. The spatio-temporal expression of PeSpr1 in the ambient air and 2% $O_2 + 30% CO_2$ storages correlated with the Spr activity. Thus, the authors concluded that PeSpr1 plays an important role in post harvested *P. eryngii*, information that is valuable for post harvest investigation.

Newly, Huang, Lin & Tsai (2015) studied the effect of ultraviolet-B (UV-B) light irradiation on the vitamin D2 content of edible fruiting bodies and mycelia of *P. eryngii*, *P. citrinopileatus* Singer, *P. ferulae* Lanzi., *P. ostreatus* and *P. salmoneostramineus* L. Vass., and their antioxidant properties. The vitamin D2 content of irradiated fruiting bodies significantly increased from 0–3.93 to 15.06–208.65 mg/g, Vitamin D2 content in irradiated mycelia of *P. citrinopileatus*, *P. ostreatus* and *P. salmoneostramineus* mushrooms increased from 0.28–5.93 to 66.03–81.71 mg/g, respectively. The three irradiated mycelium polysaccharide contents decreased from 1.3% to 24.6%. Despite
the fact that UV-B irradiation affects the content of ergothioneine, flavonoids and total phenols, the irradiated samples still contained a sufficient amount of these antioxidant components.

5. Isolated Compounds and Bioactivity

Demand is growing in the food industry for new functional ingredients or bioactive compounds from natural sources, as they are widely applied in the formulation of functional foods. This has promoted, especially in the past years, an increasing interest in extracting ingredients from foods such as mushrooms and in developing functional foods (Li & Shah, 2015).

Numerous bioactive compounds, namely polysaccharides, peptides, glycoproteins, phenolics, lipids and hydrolytic and oxidative enzymes have been extracted from crude extracts, mycelia, and basidioma of Pleurotus spp. for investigation purposes. Two of the most interesting bioactive compounds produced by Pleurotus mushrooms are the immune stimulant polysaccharides and the natural statins. The latter are hypocholesterolemic and with higher activity than the synthetic ones due to their milder side effects (Inácio et al., 2015a). Patel, Naraian & Singh (2012) published a comprehensive account on the medicinal properties of extracts of both fruiting bodies and mycelium of Pleurotus mushrooms. Their list includes antihypercholesterolemic, antihypertensive, antidiabetic, antiobesity, antiaging, antimicrobial, and antioxidant activities in addition to a hepatoprotective action. Also, different types of extracts from Pleurotus mushrooms have been reported as potential anticancer agents in several tumor cell lines, acting through distinct mechanisms. Clear clinical evidence of anticancer
activities of *Pleurotus* mushrooms, however, is still not available (Khan & Tania et al. 2012).

**Table 3** presents a compilation of the last decade most important studies on *Pleurotus* spp. mushroom fractions and isolated/identified compounds, including high (e.g. polysaccharides, small peptides and proteins) and low (e.g. terpenes, fatty acid esters and polyphenols) molecular weight compounds, as well as their corresponding bioactivities.

5.1. **High molecular weight compounds**

Several polysaccharides have been isolated from the fruiting bodies, cultured mycelia and culture filtrates of various mushrooms (Ren, Pereira & Hemar, 2012). Those polysaccharides showing antitumor activity have a great variety of chemical composition and structure, with different types of glycosidic linkages, such as (1,3)-, (1,6)- β-glucans and (1,3)- α-glucans (**Figure 3A**). In what refers to the polysaccharides from *Pleurotus* sp, Facchini et al. (2014) reported the efficacy of a polysaccharide fraction obtained from the mycelium of *P. ostreatus* with NH$_4$-oxalate at 100 °C in inhibiting the development of Ehrlich Tumor (ET) and Sarcoma 180 (S-180). Also, Llauradó et al. (2015) examined the *in vitro* antimicrobial and the complement/macrophage stimulating effects of a hot water extract from the mycelium of *Pleurotus* sp. The extract activated the microbial autolytic system of both bacterial and yeast strains, acting also on innate immunity by triggering the complement system via the alternative pathway (and presumably the classical pathway of adaptive immunity) and by enhancing macrophage functions. The authors suggested the polysaccharide-rich extract as an accessible and innovative antimicrobial food
ingredient. Recently, Li & Shah (2015) added a polysaccharide extracted from *P. eryngii* (PEPS) to milk before its fermentation process. They found that the addition of PEPS had a considerable effect on bacterial growth, texture properties, and proteolytic and ACE inhibitory activities of fermented milk during refrigerated storage and proposed its use as a nutritional and functional additive.

Zhang et al. (2014) performed the purification and measured the antioxidant activities of intracellular zinc polysaccharides (IZPS) from *P. cornucopiae*. IZPS subfractions, separated chromatographically by means of a DEAE-52 cellulose anion-exchange column, showed higher antioxidant activities *in vitro* and *in vivo*. Rhamnose and glucose were the predominant monosaccharides in IZPS, which also contains in its structure, xylose, mannose, and galactose. In turn, Li & Shah (2014) performed the sulphonation of polysaccharides from *P. eryngii* and reported that their antioxidant and antibacterial properties had improved due to the sulphonation process.

The following bioactive polysaccharides and proteins isolated/identified from *Pleurotus* spp., along the past years should be remarked: (1) a (1→3),(1→6)-linked β-glucan isolated from *P. pulmonarius* with proven anti-inflammatory and analgesic properties in a rodent model (Smiderle et al., 2008); (2) nebrodeolysin, a novel hemolytic protein isolated from *P. nebrodensis* that induced apoptosis in L929 and HeLa cells and presents anti-HIV-1 activity in CEM cell culture (Ly et al, 2009); (3) a RNase purified from *P. djamor* that inhibits the proliferation of hepatoma cells and breast cancer cells (Wu et al., 2010).

More recently, Silveira et al. (2014) made the first report of a linear (1 → 3)-β-D-glucan isolated from the fruiting bodies of *P. sajor-caju*. Its bioactivities were evaluated *in vitro*, using THP-1 macrophages, and *in vivo*, through formalin and peritonitis tests in
mice. The glucan was able to inhibit the inflammatory phase of nociception induced by formalin at a low dose and reduced the number of total leukocytes and myeloperoxidase (MPO) levels induced by LPS. Shortly after, the same group purified and identified a mannogalactan constituted by a main chain of (1→6)-linked α-D-Galp and 3-O-methyl-α-D-Galp units (Silveira et al., 2015), that was obtained from *P. sajor-caju*. The mannogalactan was able to reduce the nociception, in vivo, in the writhing and in formalin tests and reduced the carrageenan-induced paw edema, indicating that it could be an effective antinociceptive and anti-inflammatory agent.

Freshly, Cui et al. (2015) purified and characterized a novel *P. nebrodensis* polysaccharide (PN-S), and evaluated its immune-stimulating activity in RAW264.7 macrophages. They observed that PN-S effectively modulated phagocytosis levels and enhanced the immune activity of murine peritoneal macrophages. Yan, Jing & Wang (2015) also isolated and characterized a polysaccharide (PNPA) from the fruiting bodies of *P. nebrodensis*, and further examined its effect on myocardial ischemia–reperfusion (I/R) injury in rats and elucidated the underlying mechanism. The PNPA had a backbone consisting of 1,3-linked-D-glucopyranosyl and 1,3,6-linked-D-galactopyranosyl residues, which was terminated with a 1-linked-D-mannopyranosyl terminal at O-3 position of a 1,3,6-linked-D-galactopyranosyl unit along the main chain in the ratio of 4:1:1. According to the authors, PNPA exerted a protective effect on myocardial I/R injury in part through improving endogenous antioxidants and suppressing myocardial cell apoptosis. Finally, Ren et al. (2015) performed the isolation of polysaccharides (PAP) from the fruiting bodies of *P. abalonus*, and evaluated their antiproliferative activity in human colorectal carcinoma LoVo cells. HPLC analysis showed that PAP consisted of D-mannose, D-ribose, L-rhamnose, D-glucuronic acid, D-glucose and D-galactose, and that their corresponding mole percentages were 3.4%,
1.1%, 1.9%, 1.4%, 87.9% and 4.4%, respectively. The authors reported that the PAP has anti-proliferative effects against human colorectal carcinoma LoVo cells via cell cycle arrest at the S-phase and cellular apoptosis, and that the generation of ROS is a critical mediator in PAP-induced LoVo cancer cell growth inhibition.

Hagiwara et al. (2005) demonstrated the antihypertensive effect of a D-mannitol isolated from *P. cornucopiae*, through the inhibition of an angiotensin I converting enzyme (ACE), in spontaneously hypertensive rats (SHR) by oral administration. Later, in another *in-vivo* study, Jang et al. (2011) described the characterization of a new angiotensin I-converting enzyme (ACE) inhibitory peptide isolated from the basidioma of *P. cornucopiae*. In their study, two types of the purified ACE inhibitors were obtained and posteriorly analyzed. Amino acid sequences of the two purified oligopeptides were found to be RLPSEFDLSAFLRA and RLSGQTIEVTSEYLFRH. The water extracts of the *P. cornucopiae* fruiting body showed antihypertensive effect on spontaneously hypertensive rats at the dose of 600 mg/kg. Yahayaa, Rahmana & Abdullah (2014), in a recent review, reported the therapeutic potential of mushrooms in preventing and ameliorating hypertension, and listed the mostly noted *Pleurotus* species having antihypertensive effects: *P. ostreatus*, *P. cornucopiae*, *P. nebrodensis*, and *P. cystidiosus*.

### 5.2. Low molecular weight compounds

Menikpurage et al. (2009) investigated the activity of chemical components in *P. cystidiosus* against *Colletotrichum gloeosporioides*, with the purpose of developing a novel method to control anthracnose. The antifungal activity was investigated by fractionating the mushroom with acetone (A), dichloromethane (D), and hexane (H).
After antifungal assay and normal phase chromatography, the fraction with the highest inhibitory activity was separated using the Chromatotron and a single compound (A2-3-13) was isolated. Using NMR spectroscopy they found it was 3β, 5α, 6β-trihydroxyergosta-7,22-diene (Figure 3B), an oxidized ergosterol active against *C. gloeosporioides*.

Later, Suseem & Saral (2013) performed a complete analysis of the essential fatty acid esters of *Pleurotus eous* (Berk.) Sacc. and investigated its antibacterial activity. A petroleum ether extract of the *P. eous* fruiting bodies was analysed by CG-MS and 5 compounds were identified: cyclopentanetridecanoic acid, methyl ester; tartronic acid, (p-ethoxyphenyl), diethyl ester; 7, 10-Octadecadenoic acid, methyl ester; Heptadecanoic acid, 16-methyl, methyl ester and 9-Octadecenoic acid [Z]−, 2-hydroxyl-1-[hydroxymethyl] ethyl ester. Among several crude extracts tested, only the petroleum ether extract showed strong antibacterial activity by inhibiting the growth of both Gram positive and Gram negative bacterial isolates. The authors suggested that *P. eous* could be added as an extra nutrient to food products as it constitutes a new potential source of natural antibacterial agents.

Wang et al. (2013) reported the isolation, identification, and bioactivity of monoterpenoids and sesquiterpenoids isolated from the mycelia of *P. cornucopiae* (Figure 4). In their work, four new monoterpenoids (1–4) and one new sesquiterpenoid (6) were obtained from the solid culture of *P. cornucopiae* fermented on rice. Compound 1 presented an unusual spiro [benzofuran-3,2′-oxiran] skeleton. Compounds 1–5, 7, and 8 showed moderate inhibitory activity against nitric oxide production in lipopolysaccaride-activated macrophages. Compounds 6 and 7 exhibited slight cytotoxicity against HeLa and HepG2 cancer cells. Compounds 1–8 were isolated for
the first time from *P. cornucopiae*, what advances the understanding of the secondary metabolism of this fungus.

A few years ago, Lee et al. (2007) investigated the antioxidant properties of ethanolic, cold and hot water extracts prepared from *P. citrinopileatus* fruiting bodies, mycelia and fermentation filtrate. They found that all the extracts had antioxidant properties. However, three extracts from the fruiting bodies were more effective than those of the mycelia and filtrate. Ethanolic extracts were more effective as antioxidants, except for the hydroxyl radicals scavenging ability. The contents of total phenols were higher in three extracts from the fruiting bodies (8.62–12.38 mg/g). In addition, the contents of total phenols were moderately to highly (0.425–0.948 mg/g) associated with antioxidant properties.

In a recent study of our group, we investigated and compared the hydrophilic and lipophilic compounds as well as the antioxidant, anti-inflammatory and antimicrobial activities of formulations (ethanol extracts) prepared with fruiting bodies and submerged culture mycelia of *P. ostreatoroseus* Singer (Corrêa et al., 2015). We found that the bioactive formulations contain at least five free sugars, four organic acids, four phenolic compounds and two tocopherols. The fruiting body-based formulation revealed higher reducing power, DPPH scavenging activity, β-carotene bleaching inhibition and lipid peroxidation inhibition in brain homogenates than the mycelium-based preparation, as well as higher anti-inflammatory and antimicrobial activities. In addition, the absence of hepatotoxicity was confirmed in porcine liver primary cells. We concluded that these functional responses are related to the levels of bioactive components including phenolic acids, organic acids and tocopherols.
6. Concluding Remarks

In the last years, several research groups described pharmacological effects from both fruiting bodies and mycelia extracts of *Pleurotus* spp. The present review proposes that not only *Pleurotus* basidiomata but also their mycelia should being explored as a great renewable and easily accessible resource for developing functional foods/nutraceuticals and even pharmaceutical agents with antioxidant, antimicrobial, anti-inflammatory, antitumor and immunomodulatory effects. Unfortunately, precise identifications of specific molecules involved in the bioactivity of mushroom extracts are not very abundant. This is clearly an area still demanding considerable efforts. Chemically defined molecules isolated from *Pleurotus* spp may represent an exciting advance for their characterization as functional foods and as source of new innovative drugs. Further studies including clinical trials need to be carried out to ascertain the safety of these compounds as adequate alternatives to conventional drugs. The detection of novel bioactives in less explored *Pleurotus* species, together with the determination of their chemical structures and mechanisms of action, are demands that science might seek to accomplish in the near future.

Conflict of interest

The authors declare no competing financial interest.

Acknowledgements

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CAPES Foundation, Ministry of Education of Brazil (CAPES fellow, process number BEX 3974/14-6). R.M. Peralta and A. Bracht are Research Fellows of CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico).

References


Pedneault, K., Angers, P., Avis, T. J., Gosselin, A., & Tweddell, R. J. (2007). Fatty acid profiles of polar and non-polar lipids of *Pleurotus ostreatus* and *P. cornucopiae* var. 'citrino-pileatus' grown at different temperatures. Mycological Research, 111, 1228-1234.


silver nanoparticles from *Pleurotus djamor* var. *roseus* and their in vitro cytotoxicity effect on PC3 cells. Process Biochemistry, 50, 140-147.


Table 1. Main focuses of the last ten year publications and geographical distribution of most known *Pleurotus* spp*.*

<table>
<thead>
<tr>
<th>Species</th>
<th>Main areas of publications in the last decade</th>
<th>Geographical distribution</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. cornucopiae</em></td>
<td>Isolation and characterization of bioactive compounds, bioactive properties</td>
<td>Europe, Asia</td>
<td>Zhang et al. (2014), Wang et al. (2013), Jang et al. (2011), Hagiwara et al. (2005)</td>
</tr>
<tr>
<td><em>P. cystidiosus</em></td>
<td>Isolation and characterization of bioactive compounds, aroma extracts</td>
<td>Europe, Asia, North America, South America</td>
<td>Maftoun et al. (2015), Usami et al. (2014), Menikpurage et al. (2009)</td>
</tr>
<tr>
<td>Species</td>
<td>Characteristics</td>
<td>Regions</td>
<td>References</td>
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<tr>
<td><em>P. ostreatus</em></td>
<td>Isolation and characterization of bioactive compounds, bioactive properties, enzyme production, biotransformation, nanoparticles (most studied <em>Pleurotus</em> species)</td>
<td>Widespread around the world</td>
<td>Maftoun et al. (2015), El-Batal et al. (2015), Facchini et al. (2014), Purmono et al. (2013)</td>
</tr>
<tr>
<td><em>P. djamor</em></td>
<td>Bioactive properties, applications, enzyme production, nanoparticles</td>
<td>Indonesia, Malaysia, Japan, Mexico</td>
<td>Ramam et al. (2015), Velioglu &amp; Urek (2015), Wu et al. (2010)</td>
</tr>
<tr>
<td><em>P. pulmonarius</em></td>
<td>Isolation and characterization of bioactive compounds, bioactivities, enzyme production, biotransformation, spent mushroom substrate</td>
<td>Widespread around the world</td>
<td>Inácio et al. (2015b), Silveira et al. (2015), Juárez et al. (2011), Smiderle et al. (2008)</td>
</tr>
<tr>
<td><em>P. nebrodensis</em></td>
<td>Polysaccharides with bioactive properties, new technologies to improve production/extend mushroom shelf-life</td>
<td>China, Southern Europe, Central Asia</td>
<td>Yan, Jing &amp; Wang (2015), Cui et al. (2015), Lv et al. (2009), Xiong et al. (2009)</td>
</tr>
<tr>
<td><em>P. citrinopileatus</em></td>
<td>Compounds with bioactive properties, cultivation techniques, antioxidant potential</td>
<td>Asia, Southern United States, Mexico</td>
<td>Huang, Lin &amp; Tsai (2015), Kulshreshtha (2013), Liu et al. (2012), Lee et al. (2007)</td>
</tr>
</tbody>
</table>

*All species are saprotrophic and edible.*
<table>
<thead>
<tr>
<th>Species</th>
<th>Cultivation or special postharvest techniques</th>
<th>Application</th>
<th>Novelty, main contribution</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. calyptratus</em></td>
<td>Static or shaken submerged culture with N-rich and N-limited Kirk media</td>
<td>Decolourization of Orange G and Remazol Brilliant Blue R</td>
<td>Orange G decolorization in <em>P. calyptratus</em> was caused mainly by laccase, while RBBR decolorization was effected by manganese peroxidase (MnP).</td>
<td>Eichlerová et al. (2005)</td>
</tr>
<tr>
<td><em>P. dryinus</em></td>
<td>Submerged cultures with mandarin peels and tree leaves</td>
<td>Production of lignocellulolytic enzymes</td>
<td>A simple and inexpensive medium containing only mandarin peels and yeast extract as sole carbon and nitrogen sources was developed. This medium allowed simultaneous production of high levels of both hydrolases and oxidative enzymes by <em>P. dryinus</em>. By adding Mn$^{2+}$ to the medium it was possible to control the ratio between laccase and MnP.</td>
<td>Elisashvili et al. (2006)</td>
</tr>
<tr>
<td><em>P. calyptratus</em></td>
<td>Static cultivation with N-limited Kirk medium or malt extract medium</td>
<td>Decolourization of industrial dyes and enzymes production</td>
<td><em>P. calyptratus</em> was able to decolorize efficiently several synthetic dyes, especially Orange G and RBBR. A more rapid Orange G decolorization in Kirk medium was detected, while RBBR was decolorized to a higher extent in Malt extract medium. The strain produced a relatively high amount of Lac, MnP and also aryl-alcohol oxidase.</td>
<td>Eichlerová, Homolka &amp; Nerud (2006)</td>
</tr>
<tr>
<td><em>Pleurotus</em> spp.</td>
<td>Not available</td>
<td>Characterization of non-volatile</td>
<td>Four <em>Pleurotus</em> species, including <em>P. djamor</em>, <em>P. ferulae</em>, <em>P. nebrodensis</em> and <em>P. sapidus</em> were studied. Glutamic acid,</td>
<td>Guo, Lin &amp; Lin</td>
</tr>
</tbody>
</table>

Table 2. Cultivation techniques, postharvest handling and main industrial applications of *Pleurotus* spp. in the last decade.
components

Aspartic acid, leucine and arginine were the major amino acids in these four species. Their palatable amino acid contents were high in *P. ferulae*, moderate in *P. nebrodensis* and *P. sapidus*, and low in *P. djamor* (15.8 mg/g). The four *Pleurotus* species studied were distinctly different in non-volatile components.

*P. ostreatus* and *P. cornucopiae* var. *'citrino-pileatus'*

Solid state cultivation (SSC) in cottonseed hulls

Environmental manipulation of fatty acid (FA) profiles in *Pleurotus* mushrooms

Variations in the growth temperature influenced the FA profiles in both tested mushrooms. Lowering the growth temperature below 17 °C provided an expected increase in FA unsaturation in polar and non-polar lipids of *P. ostreatus*. Therefore, it may be possible to manipulate environmentally lipid unsaturation in *Pleurotus* spp. through modified growth temperature.

*Pleurotus* spp.

Submerged and solid-state fermentation in several lignocellulosic wastes

Production of lignocellulolytic enzymes

The study pointed out that the nature of lignocellulosic material and the method of fungi cultivation are factors determining the expression of lignocellulolytic potential of fungi as well as the ratio of individual enzymes in enzyme complexes. SSF of tree leaves is favorable for laccase and MnP secretion by the majority of the *Pleurotus* strains, whereas SF provides better production of hydrolytic enzymes.

*P. nebrodensis*

Postharvest irradiation with $^{60}$Co

$\gamma$-irradiation as a strategy for extending mushrooms shelf life

An irradiation dose of 1.2 kGy significantly delayed the onset of fruiting body softening, splitting and browning compared with non-irradiated controls and test samples subjected to lower or higher irradiation doses. It also had a positive effect on other indicators of mushroom tissue senescence, resulting in smaller decreases in soluble protein levels and more protracted increases in proteinase activity.

(Pedneault et al. 2007)

Elisashvili et al. (2008)

Xiong et al. (2009)
<table>
<thead>
<tr>
<th><strong>Species</strong></th>
<th><strong>Substrate</strong></th>
<th><strong>Method</strong></th>
<th><strong>Results</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. pulmonarius</em></td>
<td>SSC in pangola grass</td>
<td>Spent Mushroom Substrate (SMS) used in treatment of chlorothalonil containing wastewater</td>
<td>Freshly obtained SMS extract was able to reduce 100% of the initial concentration of chlorothalonil (2 mg/l) after 45 min of reaction. Storage time had a negative effect on the stability of the enzymatic activity. Cooling and freezing the SMS extract also had a negative effect on chlorothalonil degradation. Juárez et al. (2011)</td>
</tr>
<tr>
<td><em>P. citrinopileatus</em></td>
<td>SSC in handmade paper and cardboard industrial wastes</td>
<td>A sustainable and green proposal for mushroom cultivation</td>
<td><em>P. citrinopileatus</em> was cultivated on a sludge of handmade paper and cardboard industrial waste. Protein content, carbohydrate content and fat content of all carpophores were found to significantly decrease over control. Besides, carpophores were found to possess frameshift mutagens from the sludge. However, the use of a combination of sludge and wheat straw not only increased the biological efficiency but also provided less mutagenic carpophores. Kulshreshtha et al. (2013)</td>
</tr>
<tr>
<td><em>P. eryngii</em></td>
<td>SSC in casing materials</td>
<td>A sustainable and green proposal for mushroom cultivation</td>
<td>Enhanced yield of <em>P. eryngii</em> was achieved on spent compost casing material. Use of casing materials enhanced the yield by 21–107% over non-cased substrate. Casing of substrate using locally available materials to maximise bioconversion efficiency of <em>P. eryngii</em> constitutes a relatively easy, feasible and low-cost practice. Mishra et al. (2013)</td>
</tr>
<tr>
<td><em>P. ostreatus</em></td>
<td>Not available</td>
<td><em>P. ostreatus</em> nano-particles as a new nano-biosorbent</td>
<td>The use of <em>P. ostreatus</em> nano-particles (PONP) as a new nano-adsorbent to remove Mn(II) from aqueous solution was investigated. The maximum Mn(II) adsorption capacity of PONP was 130.625 mg/g at 298.15 K, which was higher than many other adsorbents. Ma et al. (2013)</td>
</tr>
<tr>
<td><strong>Species</strong></td>
<td><strong>Culture Medium</strong></td>
<td><strong>Biotransformation of</strong></td>
<td><strong>Description</strong></td>
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</tr>
<tr>
<td><em>P. ostreatus</em></td>
<td>Liquid stationary cultures with potato dextrose broth</td>
<td>Biotransformation of synthetic insecticide</td>
<td>The ability of <em>P. ostreatus</em> to transform heptachlor as well as heptachlor epoxide was investigated. Heptachlor was eliminated by this fungus in PDB and HN media during a 14d incubation period. <em>P. ostreatus</em> was also capable of degrading heptachlor epoxide, which is a recalcitrant metabolite of heptachlor.</td>
</tr>
<tr>
<td><em>P. pulmonarius</em></td>
<td>Seed culture in brown-sugar:ricebran:malt medium (BRMY) and SSC in rubber wood sawdust</td>
<td>Optimization of mushroom commercial cultivation</td>
<td>A high amount of <em>P. pulmonarius</em> liquid spawn was produced in BRMY medium using an automated bioreactor. High yield, uniform, small pellets were obtained in just three days. The liquid inoculum had the ability to colonise sterile rubber wood sawdust as fruiting substrates in a shortened time suggesting that the mycelium was dispersed more efficiently as opposed to grainspawn.</td>
</tr>
<tr>
<td><em>P. cystidiosus</em></td>
<td>Liquide culture of basidioma in Sabouraud dextrose broth (SDB)</td>
<td>Production of bioactive lipids</td>
<td>This innovative study reports the successful cultivation of mushrooms in liquid medium. SDB was the most suitable culture medium and the maximal mycelial biomass of <em>P. cystidiosus</em> was obtained in SDB at pH 7, when incubated at 28°C and 30°C. Agitation did not improve mycelial growth. Cholesterol, triglycerides, free fatty acids, and polar lipids were detected in <em>P. cystidiosus</em> mushrooms.</td>
</tr>
<tr>
<td><em>P. eryngii</em> var. <em>tuoliensis</em> and <em>P. cystidiosus</em></td>
<td>Not available</td>
<td>Characterization of odor components of the volatile oil from <em>Pleurotus</em> spp.</td>
<td>The main components of the <em>P. eryngii</em> var. <em>tuoliensis</em> oil were palmitic acid, oleic acid and linoleic acid. The main components of the <em>P. cystidiosus</em> oil were palmitic acid, indole and myristic acid. The results of the sniffing test, odor activity value (OAV) and flavor dilution (FD) factor indicate that methional, 1-octen-3-ol and nonanal are the main aroma-active</td>
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</table>
components of *P. eryngii* var. *tuoliensis* oil, while dimethyl trisulfide and 1-octen-3-ol were estimated as the main aroma-active components of the *P. cystidiosus* oil.

This study assessed the performance of mushroom initiation and yield by cold stimulation of *P. pulmonarius*. Various combinations of temperature and time were examined in a factorial design, in order to determine the most appropriate cold stimulation treatment. The best performance among the 12 treatments was recorded following a 12 h cold stimulation at 5°C.

The high activity of serine proteinase (Spr) was one of the key factors causing deterioration of mushroom fruiting bodies. To investigate the activity and molecular mechanisms of Spr during storage in *P. eryngii*, the mushrooms were stored under high carbon dioxide and low oxygen treatment (2% O₂ + 30% CO₂), which was proved to significantly prolong mushroom shelf life.

Optimization of production conditions yielded an enzyme with activity over 32,450 IU/g of fermented substrate. Factorial design was capable of establishing the conditions that multiplied the activity of the enzyme several fold. The partially purified enzyme was capable of decolorizing several dyes with over 80% reduction in color. The enzyme was also used in the synthesis of gold nanoparticles.
<table>
<thead>
<tr>
<th><strong>P. djamor var. roseus</strong></th>
<th>SSC in paddy straw substrate</th>
<th>Mycosynthesis and characterization of silver nanoparticles</th>
<th>The present study reports the biological synthesis of silver nanoparticles (AgNPs) using an aqueous extract of <em>P. djamor</em> var. <em>roseus</em> and its cytotoxicity against human prostate carcinoma (PC3) cells. Nanoparticle formation was confirmed by UV–visible (UV–vis) spectroscopy, transmission electron microscopy (TEM), X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FTIR) analysis.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>P. eryngii</strong></td>
<td>SSC in several lignocellulosic wastes</td>
<td>Strategy for extending the shelf life and improving yield</td>
<td>Among all tested mushroom medias, the one that produced the longest shelf life and highest yield contained 4.5% of crude protein and 15% of nitrogen free extracts. Regression analysis supported CP and CaO presented synergistic effects on shelf life. These results might be used by mushroom farmers to produce long shelf life and high yield mushrooms.</td>
</tr>
<tr>
<td><strong>P. djamor</strong></td>
<td>SSF with various industrial wastes</td>
<td>Optimization of cultural conditions for biosurfactant production</td>
<td>This study demonstrated an economical biosurfactant production by <em>P. djamor</em> in SSF in determined the optimum condition. In this condition 10.205 g/l biosurfactant was produced which reduced water surface tension to 28.82 mN/m. In laboratory’s large-scale production 8.9 g/l biosurfactant was produced, which was carried out in a tray bioreactor. With regard to dual product strategies, a lipase enzyme was simultaneously produced.</td>
</tr>
<tr>
<td><strong>P. florida and P. flabellatus</strong></td>
<td>SSC in agro-residues combined with biogas digester residue</td>
<td>Cultivation on a combination of anaerobically digested plant material and</td>
<td>The study investigated the effects of the addition of biogas digester residue (BDR) to paddy straw (PS) and coir pith (CP), used as substrates for growing mushroom. The substrate that produced higher yields and biological efficiency was PS mixed with BDR followed by CP with BDR. Addition of BDR with</td>
</tr>
<tr>
<td>Species</td>
<td>Substrate</td>
<td>Methodology</td>
<td>Result/Description</td>
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<tr>
<td><em>P. pulmonarius</em></td>
<td>SSC in orange waste</td>
<td>Enzymes production and biotransformation of orange waste</td>
<td>Pectinase was the main hydrolytic enzyme produced by the fungus, with the highest enzymatic activity of 9.4 U/mL after 35 days of cultivation. Laccase was the main oxidative enzyme produced with maximal activity of 12.2 U/mL obtained after 20 days of cultivation. There was no lignin degradation during the cultivation and the fungus culture promoted a protein enrichment in the substrate.</td>
</tr>
<tr>
<td><em>P. ostreatus</em></td>
<td>SCC in blank and printed paper substrates</td>
<td>A sustainable proposal for mushroom cultivation and a profitable means to recycle paper</td>
<td>The objective of this work was to evaluate the chemical composition of fruiting bodies of <em>P. ostreatus</em> grown on blank and printed paper substrates, in comparison with samples grown on oat straw (control). The nutritional properties of the control sample were similar to values reported in the literature, while the chemical composition of the samples obtained using paper scraps, either blank or printed, was highly satisfactory.</td>
</tr>
<tr>
<td><em>P. ostreatus</em></td>
<td>Not available</td>
<td>Improvement of antioxidant ability and rheological properties in yogurts</td>
<td>The multiplication of fermentative bacteria was greater in yogurts supplemented with <em>P. ostreatus</em> aqueous extract (POE). The utilization of POE in yogurts improves rheological properties and texture characteristics (lower firmness but higher cohesiveness, adhesive, springiness and less syneresis). The supplemented yogurts with POE contained more total phenolics and exhibited higher antioxidant activity than controls.</td>
</tr>
</tbody>
</table>
Table 3. Chemical compounds in *Pleurotus* spp. and their correspondent bioactivities reported in the past ten years.

<table>
<thead>
<tr>
<th><em>Pleurotus</em> spp.</th>
<th>Compound and Bioactivity</th>
<th>Novelty, main contribution</th>
<th>Ref.</th>
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</thead>
<tbody>
<tr>
<td><em>P. cornucopiae</em></td>
<td>D-mannitol, ameliorates hypertension</td>
<td>The antihypertensive effect, induced by the inhibition of an angiotensin I converting enzyme (ACE), was demonstrated in spontaneously hypertensive rats (SHR) by oral administration.</td>
<td>Hagiwara et al. (2005)</td>
</tr>
<tr>
<td><em>P. citrinopileatus</em></td>
<td>Polyphenols, antioxidant effect</td>
<td>The ethanolic, cold and hot water extracts of <em>P. citrinopileatus</em> fruiting bodies, mycelia and fermentation filtrate were evaluated for their antioxidant properties. Overall, extracts from fruiting bodies presented a superior antioxidant potential than those from mycelia and filtrate.</td>
<td>Lee et al. (2007)</td>
</tr>
<tr>
<td><em>P. pulmonarius</em></td>
<td>β-glucan, anti-inflammatory and analgesic properties</td>
<td>A glucan extracted from the basidioma was tested for its effects on the acetic acid-induced writhing reaction in mice, a typical model for quantifying inflammatory pain. The great anti-inflammatory and analgesic activities observed were possibly by the inhibition of pro-inflammatory cytokines.</td>
<td>Simiderle et al. (2008)</td>
</tr>
<tr>
<td><em>P. nebrodensis</em></td>
<td>Nebrodeolysin, antitumoral and anti-HIV-1 effects</td>
<td>A novel hemolysin was isolated from <em>P. nebrodensis</em> by ion exchange and gel filtration chromatography. It exhibited haemolytic activity towards rabbit erythrocytes and caused efflux of potassium ions from erythrocytes, with strong cytotoxicity against Lu-04, Bre04, HepG2, L929 and HeLa cells, besides anti-HIV1 activity in CEM cell culture.</td>
<td>Lv et al. (2009)</td>
</tr>
<tr>
<td><strong>Species</strong></td>
<td><strong>Activity</strong></td>
<td><strong>Details</strong></td>
<td><strong>References</strong></td>
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<td>---------------------</td>
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</tr>
<tr>
<td><em>P. cystidiosus</em></td>
<td>Ergosterol, antifungal activity</td>
<td>The antifungal activity was investigated by fractionating the mushroom to acetone (A), dichloromethane (D), and hexane (H). After antifungal assay and normal phase chromatography, the fraction with the highest inhibitory activity was separated using the Chromatotron and a single compound (A2-3-13) was isolated. Using NMR spectroscopy they found it was 3β, 5α, 6β-trihydroxyergosta-7,22-diene.</td>
<td>Menikpurage et al. (2009)</td>
</tr>
<tr>
<td><em>P. djamor</em></td>
<td>Ribonuclease, antiproliferative activity</td>
<td>A 15-kDa RNase was purified from <em>P. djamor</em> using ion exchange chromatography and gel filtration. The RNase exhibited maximal RNase activity at pH 4.6 and 60 °C. It inhibited proliferation of hepatoma cells and breast cancer cells.</td>
<td>Wu et al. (2010)</td>
</tr>
<tr>
<td><em>P. sajor-caju</em></td>
<td>Polysaccharides, antineoplastic Effect</td>
<td>Female Swiss mice were inoculated with the Ehrlich ascitic tumor and the polysaccharidic fractions of <em>P. sajor-caju</em> were administered intraperitoneally, during a 6-day period. Two fractions presented a lower volume of ascitic liquid and a higher reduction in the number of neoplastic cells, when compared to the positive control. Glucose was the major component detected in the fractions, followed by galactose and mannose.</td>
<td>Dalonso et al. (2010)</td>
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<td><em>P. cornucopiae</em></td>
<td>Peptide, anti-hypertensive effects</td>
<td>This study describes the characterisation of a new angiotensin I-converting enzyme (ACE) inhibitory peptide from the basidioma of <em>P. cornucopiae</em>. Two types of the purified ACE inhibitors were obtained and posteriorly analysed, showing two types of oligopeptides. The amino acid sequences of the two purified oligopeptides were found to be RLPSEFDLSAFLRA and RLSGQTIEVTSEYLFRH. Water extracts of <em>P. cornucopiae</em> fruiting body showed antihypertensive effect on spontaneously hypertensive rats at a dosage of 600 mg/kg.</td>
<td>Jang et al. (2011)</td>
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<td><strong>P. cornucopiae</strong></td>
<td>Monoterpenoids and sesquiterpenoids, anti-inflammatory and antitumoral potential</td>
<td>Four new monoterpenoids (1–4) and one new sesquiterpenoid (6) were isolated from the mycelia fermented on rice. Compound 1 possesses a spiro[benzofuran-3,2'-oxiran] skeleton. The absolute configuration of the 6,7-diol moieties in compounds 1, 2, and 6 was assigned. Compounds 1–5, 7, and 8 showed inhibitory activity against nitric oxide production in lipopolysaccaride-activated macrophages while compounds 6 and 7 exhibited cytotoxicity against HeLa and HepG2 cells.</td>
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<td><strong>P. eous</strong></td>
<td>Fatty acid esters, antibacterial activity</td>
<td>Petroleum ether extract of the <em>P. eous</em> fruiting bodies were analysed by CG-MS and 5 compounds were identified: cyclopentanetridecanoic acid, methyl ester; tartronic acid, (p-ethoxyphenyl), diethyl ester; 7, 10-octadecadenoic acid, methyl ester; heptadecanoic acid, 16-methyl, methyl ester and 9-octadecenoic acid [Z]-2-hydroxyl-1-[hydroxymethyl] ethyl ester. Among several crude extracts tested, only the petroleum ether extract showed strong antibacterial activity by inhibiting the growth of both gram positive and gram negative bacterial isolates.</td>
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<td><strong>P. cornucopiceae</strong></td>
<td>Polysaccharides, antioxidant activities in vitro and in vivo</td>
<td>Intracellular zinc polysaccharides (IZPS) were extracted and purified, and three subfractions (IZPS-1, IZPS-2, and IZPS-3) were separated by anion-exchange column chromatography. They showed certain scavenging effects on superoxide anion (O2•−) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals, and positive rising of reducing power <em>in vitro</em>. All the subfractions were found able to act as upregulators of the superoxide dismutase, GSH peroxidase and catalase and significantly decreased the contents of malondialdehyde and lipid peroxidation <em>in vivo</em>.</td>
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<td><strong>P. eryngii</strong></td>
<td>Sulphated polysaccharides, antioxidant and antibacterial activities</td>
<td>Polysaccharides from <em>P. eryngii</em> (PEPS) and exopolysaccharides from <em>Streptococcus thermophilus</em> ASCC 1275 (ST1275 EPS) were sulphated, with degrees of sulphonation of 0.69 and 0.31, respectively. Antioxidant activities of both PEPS and ST1275 EPS were significantly improved after sulphonation.</td>
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*Wang et al. (2013)*

*Suseem & Saral (2013)*

*Zhang et al. (2014)*

*Li & Shah et al. (2014)*
Overall, sulphated PEPS presented a superior antibacterial potential when compared with sulphated ST1275 EPS.

The mostly noted species having antihypertensive effects include *P. ostreatus*, *P. cornucopiae*, *P. nebrodensis*, and *P. cystidiosus*. Their ameliorating effect on elevated blood pressure has been attributed to their inhibitory effect on angiotensin converting enzyme (ACE).

*P. sajor-caju* fruiting bodies cultivated in banana straw, produced a linear β-D-glucan (1→3)-linked. This is the first report of such a structure isolated from the *Pleurotus* genus. An immunomodulatory effect was observed when THP-1 macrophages were treated with the β-D-glucan. Also, the β-D-glucan was able to inhibit the inflammatory phase of nociception induced by formalin in a low dose and reduced the number of total leukocytes and myeloperoxidase (MPO) levels induced by LPS.

The efficacy of polysaccharidic fractions extracted from the mycelial biomass of *P. ostreatus* DSM 1833 in inhibiting the development of Ehrlich Tumor (ET) and Sarcoma 180 (S-180) was tested. The fraction obtained by extraction with NH₄-oxalate at 100 °C, for 3 h, 4 times, was the one that presented the best results, being effective against both tumors and, at the concentration of 30 mg/kg, showed no toxic effects on healthy animals.

The extract activated the microbial autolytic system of eight strains: seven autolyzing strains with intensity values ranging from 2.7% in *Candida* sp. to 36.1% in *Saccharomyces cerevisiae*, while autolysis was of 1.8% in one non-autolyzing strain (*Bacillus cereus*). The extract (5–100 µg/well) enhanced the acid phosphatase activity in murine peritoneal macrophages by 133–184% compared to controls. The findings introduce a novel “bifunctional” approach
The use of edible fungi has not been explored for the production and delivery of low cost vaccines, despite these organisms’ attractive features. These include the fact that edible biomass can be produced at low costs in a short period of time, its high biosynthetic capacity, its production of immunomodulatory compounds, and the availability of genetic transformation methods. Perspectives associated to this biotechnological application are identified and discussed in this review that proposes *Pleurotus* fungus as a convenient host for the development of innovative vaccines.

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<th><strong>Pleurotus spp.</strong></th>
<th><strong>β-glucans, immunomodulatory activity</strong></th>
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<tr>
<td><em>P. sajor-caju</em></td>
<td>Exopolysaccharide (EPS), anti-inflammatory activity</td>
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<tr>
<td><em>P. nebrodensis</em></td>
<td>Polysaccharide, immune-stimulating activity</td>
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(antimicrobial-immunomodulatory) to the nutraceutical potential of the *Pleurotus* hot-water mycelial extract.

The mannogalactan was purified by freeze-thawing and dialysis, and it was characterized by GC-MS analysis and NMR spectroscopy as a main chain of (1→6)-linked α-D-Galp and 3-O-methyl-α-D-Galp units. This is the first report of a methylated polysaccharide on EPS of *P. sajor-caju*. The mannogalactan was able to reduce the nociception, *in vivo*, in the writhing and formalin tests and also reduced the carrageenan-induced paw edema, which indicates that it could be an antinociceptive and anti-inflammatory agent.

A novel *P. nebrodensis* polysaccharide (PN-S) was purified and characterized, and its immune-stimulating activity was evaluated in RAW264.7 macrophages. After exposure to PN-S, the phagocytosis of the macrophages was significantly improved. PN-S treatment enhanced the productions of interleukin-6 (IL-6), nitric oxide (NO), interferon gamma (INF-γ), and tumor necrosis factor-α (TNF-α) in the macrophages, with up-regulation of mRNA expressions of interleukin-6 (IL-6), inducible nitric oxide synthase (iNOS), interferon gamma(INF-γ) and tumor necrosis factor-α (TNF-α) being observed in a dose-dependent manner, as

Pérez-Martínez et al. (2015)

Silveira et al. (2015)

Cui et al. (2015)
A polysaccharide (PNPA) from the fruiting bodies of *P. nebrodensis* was isolated, characterized and the effect of PNPA on myocardial ischemia–reperfusion (I/R) injury in rats was further investigated. Pretreatment with PNPA for 30 days attenuated myocardial infarct size as compared to I/R model group. A decrease in superoxide dismutase, catalase and glutathione levels, as well as an increased malondialdehyde content were observed in both myocardial serum and tissues of control I/R group, whereas pretreatment with PNPA markedly restored these changes, and also relieved myocardial cell apoptosis.

Polysaccharides (PAP) from the fruiting bodies of *P. abalonus* were isolated, and the antiproliferative activity of the polysaccharides in human colorectal carcinoma LoVo cells were evaluated. HPLC analysis showed that PAP consisted of D-mannose, D-ribose, L-rhamnose, D-glucuronic acid, D-glucose and D-galactose. PAP was shown to exert a high antioxidant activity *in vitro* and a dose-dependent antiproliferative effect against LoVo cancer cells. Flow cytometry analysis demonstrated that PAP exhibited a stimulatory effect on apoptosis of LoVo cells, and induced the cell-cycle arrest at the S phase.
Figure 1. Number of research articles and reviews, and patents published in the period from 1985 to 2015 regarding the *Pleurotus* genus (obtained on Web of Science, August 2015; keyword restrict to the title: *Pleurotus*)
Figure 2. Distribution of research articles reviews and patents published in the period from 1985 to 2015 regarding the *Pleurotus* genus according to the main studied areas (obtained on Web of Science, August 2015; keyword restrict to the title: *Pleurotus*).
Figure 3. (A) Polysaccharide repeating unit purified from fruiting bodies of Pleurotus citrinopileatus (B). An oxidized ergosterol 3β, 5α, 6β-trihydroxyergosta-7,22 diene, identified in Pleurotus cystidiosus acetone dichloromethane extract. The chemical structures were drawn using the ChemWindow software (Soft Shell International Ltd) based on originals presented by Liu et al. (2012) (panel A) and Menikpurage et al. (2009) (panel B).
Figure 4. Monoterpenoids (A) and sesquiterpenoids (B) from the mycelia of *Pleurotus cornucopiae* ethyl acetate extract. Details of biological activities of compounds (1-8) are described in the text. The chemical structures were drawn using the ChemWindow software (Soft Shell International Ltd) based on originals presented by Wang et al. (2013).