Omic Tools to Study Enzyme Production from Fungi in the *Pleurotus* genus

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Fungi from the *Pleurotus* genus secrete different enzymes, including laccases, manganese peroxidases, versatile peroxidases, glycosyl hydrolases, peptidases, and esterases/lipases. This genus contains white-rot fungi, which degrade the components of plant materials. The secreted enzymes have great application in the biotechnology field. The general conditions of a fungal culture have a direct effect on the regulation of protein expression, which changes the composition of the transcriptome, proteome, and secretome. Studies have shown that the culture type, either solid or submerged, also changes the transcriptional profiles. The knowledge of the transcriptome and proteome allows one to find valuable enzymes to obtain portable fuels from lignocellulosic materials and provide information oriented to improve the enzymes production through different culture conditions. Additionally, research has been conducted on the *Pleurotus* genus to better understand its biology. Numerous tools have been used for this purpose, such as classical recombination, genetic engineering, and omic tools. The information generated by the omic sciences (comparative genomics, transcriptomics, proteomics, and metabolomics) and through bioinformatics (massive data analysis), among other things, can greatly contribute to improving production processes and the use of metabolites. This review discussed some works where omic tools have been used to study enzyme production of fungi of the *Pleurotus* genus.

Keywords: Pleurotus; Transcriptome; Proteome; Secretome; Metabolome; Enzymes

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INTRODUCTION

Fungi have made an enormous contribution to human lives and are of commercial relevance in the production of fermented products, antibiotics, enzymes, pigments, organic acids, *etc*., which have great applications in the biotechnology field. There is a great diversity of fungi and even more of their metabolites (Ferreira *et al*. 2016). Among the mushrooms of great importance are those in the genus *Pleurotus*. Paul Kummer defined the genus *Pleurotus* (Fries) Kummer (Basidiomycota, Agaricales) in 1871. They are classified as white-rot fungi and are cultivated edible mushrooms. They also have medicinal properties and many environmental and biotechnological applications. The fungi in this genus are the most cultivated mushroom. Their taxonomic classification is complicated because they have many morphological similarities that have been considered part of the taxonomic criteria (Vilgalys *et al*. 1993; Zervakis and Balis 1996). Recently, the use of molecular tools based on DNA has helped to clarify the taxonomic classification of fungi in the genus *Pleurotus* (Pawlik *et al*. 2012; Shnyreva *et al*. 2012; Avin *et al*. 2014).

With the help of molecular techniques, researchers are still searching for fungal molecules with biological activities as well as the optimum conditions necessary for their industrial production, the above is expensive and involves a lot of work in different areas of study, due to the wide variety of fungi. Therefore, omic tools can address the functions of numerous genes (functional genomics) (Bunnik and Le Roch 2013), compare genes with another organism (comparative genomics) (Haubold and Wiehe 2004), study expression profiles (transcriptome) (Wang *et al*. 2009), study expression profiles of proteins (proteome) (Zhu *et al*. 2003), and study profiles of low molecular weight metabolites present in a cell under a given set of physiological conditions (metabolomics) (Kell *et al*. 2005). The results obtained through these techniques have contributed greatly to the understanding of the interactions between environmental factors, genetic variants, genetic expression patterns, changes in the concentration of metabolites, *etc*. This has been an important scientific development and has reduced bioprocessing times and increased yields, while managing to reduce the costs of biotechnological applications. Information generated by omic tools can be analyzed through mathematical and computational models to determine the real behavior of fungal cells in response to some development conditions. This is called systems biology (Fig. 1). These results allow for a better idea of certain biological processes, which can improve the processes or production yields of metabolites of interest.

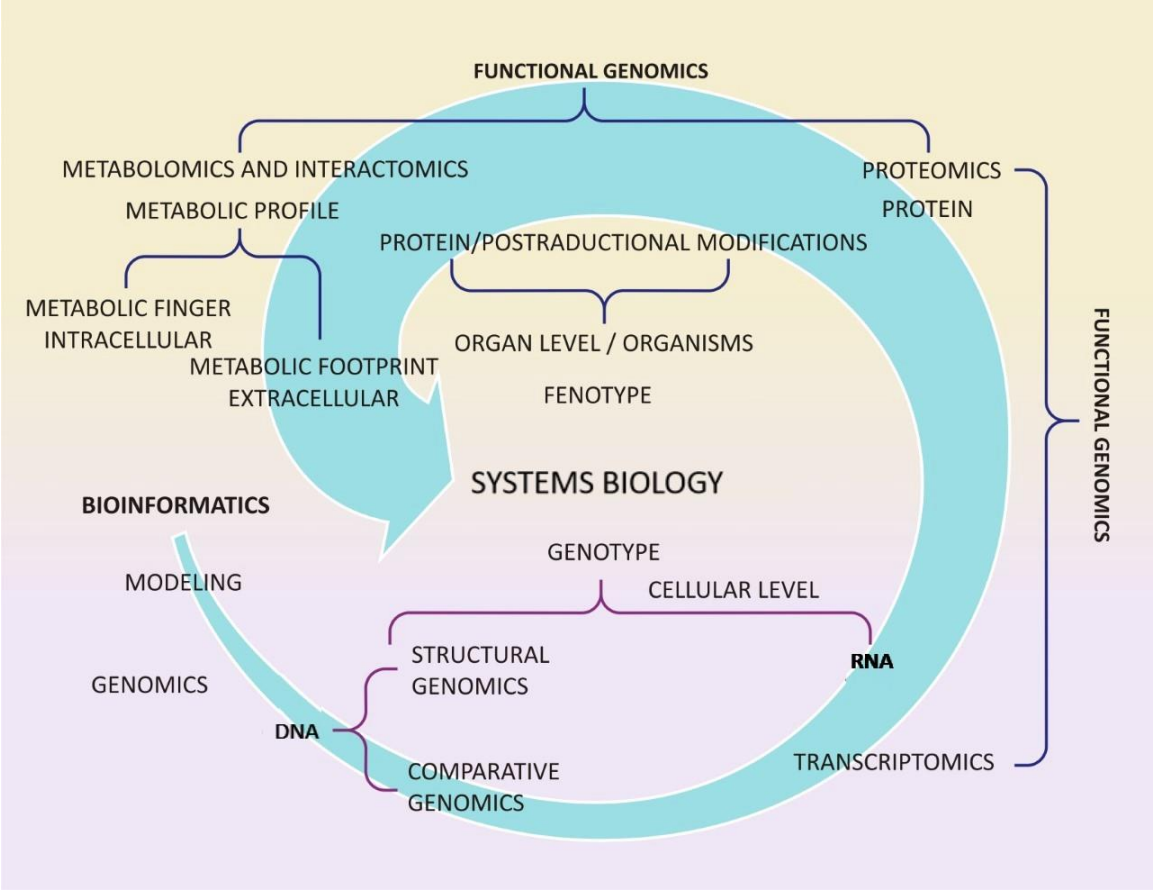

Fig. 1. Set of molecular tools and techniques that support systems biology

It is known that the successful survival of a species depends mainly upon two criteria: 1) being in a suitable environment and 2) the ability to adapt to new environmental conditions. The first criterion promotes inbreeding that maintains genetic homogeneity within a population, which can be a disadvantage because it allows the expression of mutations. In contrast, the second criterion promotes genetic heterogeneity and genetic recombination. Fungi are advantageous for use as model organisms because they are eukaryotic, which have characteristics that have helped to elucidate many cellular processes (Whiteway and Bachewich 2005). In heterothallic fungi, sexual reproduction is controlled by incompatibility factors. Bifactorial fungi have two incompatibility factors that together control mating competition. These factors are genetically unlinked, and their independent segregation in meiosis leads to the production of four kinds of progeny (Koltin *et al*. 1972). Laboratory strains typically have stable mating types and are heterothallic. Most wild strains are homothallic, but do not have stable mating types. During mitotic growth, cells can change their mating type (Whiteway and Bachewich 2005).

Pleurotus genus

The genus *Pleurotus* is bifactorial heterothallic with a tetrapolar system and multiple alleles. Fertilization is only performed between two homocariotic mycelia of different genotypes for two genes or factors (A and B) (Zervakis and Balis 1996). In a fruiting body (dikaryotic), spores are formed with a set of chromosomes (*n*). Spores give rise to a monokaryotic mycelium when germinating and a dikaryotic mycelium (plasmogamy) through the fusion of two sexually compatible monokaryotic mycelia (different alleles of incompatibility). Ramírez *et al*. (2000) reported that locus A behaves as one, whereas locus B is a complex of two genes (matBα and matBβ) linked with genetic distances ranging from 17.5 cM to 5.0 cM. Specificities can appear by recombination between the two loci, which occurs in other superior basidiomycetes. Nine different types of mating were found for A and 15 for B, some of which were the result of intra-factorial recombination (Ramírez *et al*. 2000). A dikaryotic strain is formed when two compatible monokaryotic strains mate, which results in plasmogamy, but not cariogamy. As new hyphae grow, the two nuclei divide synchronously, and each new compartment maintains two nuclei and cariogamy only occurs before the start of sexual reproduction through fusion of the nuclei (Stajich *et al*. 2009). Hyphae are involved in morphogenesis, its integrity, synthesis, and degradation, based on the environmental and physiological conditions and availability of nutrients. The hyphae from the genus *Pleurotus* contain two nuclei that are called dikaryons, with each derived from a different father. However, the patterns of gene expression for dikaryons are not well known. *Pleurotus* is a well-known genus that belongs to the Pleurotaceae family of the order Agaricales. The name *Pleurotus* comes from the Greek "pleuro", which means formed laterally or in a lateral position. This is in reference to the position of the stipe compared with that of the pileus. There are many species of this genus, of which some are cultivated to be commercialized and/or consumed. Some of the most representative fungi are *P. ostreatus*, *P. eryngii*, *P. citrinopileatus*, and *P. djamor* var. *roseus* (Fig. 2).

There have been major problems in the classification of the genus *Pleurotus* based only on the morphological characteristics (often not reliable or not blunt due to the influence of environmental conditions) or experiments on compatibility (supported by the concept of biological species) (Zervakis *et al*. 2001). Molecular tools have provided more precise methodologies for identification, one of which is the printing of DNA fingerprints. Gonzalez and Labarere (2000) related 16 isolates of *Pleurotus* spp. using the domains V4,

V6, and V9 from the rRNA of the small subunit of the mitochondria and related it to its hyphal system.

Fig. 2. Fruiting bodies of *Pleurotus* fungi: a) *P. eryngii*, b) *P. citrinopileatus*, c) *P. ostreatus* (gray), d) *P. ostreatus* (white), e) *P. djamor* var. *roseus*, and f) *P. djamor* var. *roseus* (wild)

The phylogenetic analysis using these mitochondrial sequences coincided with the classification made with morphological characteristics, nuclear genome, or isoenzymes. These studies have shown that *P. ostreatus* and *P. florida* are the same species, as well as *P. sajor-caju* and *P. pulmonarius*. They were located as phylogenetically close to the species *P. ostreatus*, *P. pulmonarius*, *P. flabellatus*, *P. sapidus*, *P. columbinus*, and *P. eryngii*, which present a monomitic hyphal system (Singer 1986). The other group contained *P. citrinopileatus*, *P. eosmus*, *P. dryinus*, and *P. smithii*, which present a dimitic hyphal system. Avin *et al*. (2017) used primers coupled with cDNA analysis to examine the diversity of sequences of the mitochondrial gene and cytochrome C oxidase subunit I (COI; universal DNA barcode for members of the animal kingdom) in six *Pleurotus* spp. (*P. ostreatus*, *P. eryngii*, *P. pulmonarius*, *P. giganteus*, *P. citrinopileatus*, and *P. djamor*) and compared them with a ribosomal internal transcribed spacer (ITS). Through the ITS, the six species were discriminated, but some sequence results could not be interpreted because of the length variation between the ITS copies. In contrast, complete COI sequences were recovered from most strains, except for three individuals of *P. giganteus* (region 5'). The COI sequences allowed the resolution of all of the species when the partial data for *P. giganteus* was excluded. Therefore, it was suggested that COI can be a useful barcode marker for fungi when cDNA analysis is adopted, which allows for identification in cases where ITS cannot be recovered and offers higher resolution when there is fresh tissue.

It has been reported that the environment has a great influence on the phenotype of fungi, which is isolated by internal homeostatic systems, so that the response of each of the cells is independent. Phenotypic traits can be classified into two groups: qualitative and

quantitative. The first type is controlled by a few or unique genes. Their inheritance is simple. They follow the Mendelian rules and the effect of the environment on them is relatively reduced (phenotypes produced by these genes are derived from the information on genotypes). However, quantitative traits are controlled by a high number of genes that are individually inherited and highly influenced by the environment (Mackay 2001).

PRODUCTION AND EXPRESSION OF ENZYMES FROM *Pleurotus* spp.

Fungi present a wide variety of functional proteins that have different roles in the acquisition of nutrients and protection against nearby organisms or unfavorable environmental conditions, among which are carbohydrases, proteases, lipases, and oxidoreductases (Kim *et al*. 2016). Depending upon the lifestyle and ecological niche, saprophyte mushrooms secrete a large number of hydrolases (mainly those that catalyze the degradation of carbohydrates) encoded by multigene families to feed on complex molecules in the environment (Lowe and Howlett 2012). Differences have been shown in the level of expression of the fungal genes involved in the degradation of lignocellulose that depend upon the culture conditions because under natural growth conditions that are more complex, the expression of these genes and the enzymatic activities could be affected by multiple factors. Many fungi species are adapted for the destruction and assimilation of wood, which is of great ecological importance because it is possible to recycle a large amount of lignocellulosic organic matter thanks to these organisms, using an enzymatic system for the biodegradation of polysaccharides and an enzymatic system for the oxidation of lignin. *Pleurotus* spp. are known to be efficient decomposers of lignocellulosic waste *via* the enzymatic system that performs selective delignification of hard and soft woods. This implies that lignin and hemicellulose are initially degraded, followed by cellulose, and most of the cellulose can remain intact (Santoyo *et al*. 2008). In many cases, the wood continues to maintain its regular crystalline structure or have regular holes. A discoloration to white occurs in many cases, but also red or green discolorations have been observed due to the remains of polyphenols. This pattern is a characteristic of some Basidiomycetes only, as is the case for *Pleurotus* spp. (Schmidt 2006). Given the catalytic capacities of many of the enzymes produced by *Pleurotus* spp., there is great interest in their study as they have important biotechnological applications (Table 1).

Fungi have been used for several years to produce enzymes. Currently, the complete genomes of many organisms are available, which has facilitated the search and production of enzymes with specific characteristics for biotechnological processes of interest (Sudhakar *et al*. 2018). Edible mushrooms have also been used as a source of enzymes and because many of them are saprobic organisms, they produce a wide range of extracellular enzymes that allow them to degrade complex lignocellulosic substrates into soluble substances; it has been suggested that edible mushroom proteins are safe and highly nutritional (Altaf *et al*. 2016). Hydrolases are found within the enzymatic system of white-rot fungi enzymes and gradually degrade cellulose. For example, endoglucanases that catalyze degradation of cellulose in amorphous regions and cellobiohydrolases that are exocellulases and produce disaccharides (cellobiose) from the reducing (type I) or non-reducing end (type II) of the cellulose, with the final degradation of cellobiose performed by β-glucosidases.

Table 1. Enzymes of *Pleurotus* spp. with Biotechnological Importance

Enzymes	Biotechnological application	References
Amylases	Amylases are classified into α, β, and γ subtypes. These enzymes are mainly used in partial or total hydrolysis of starch to produce dextrins and glucose rich syrups, respectively; the action of the amylases reduces the viscosity of the starch solution used in the coating of paper. They delay the staling in baking industry; are used as a supplement to improve digestion. They produce syrups rich in maltose, solutions with anti-inflammatory activity, help clarify juices, and very important their participation in the malting process of barley prior to alcoholic fermentation in beer production.	Tellez-Tellez *et al*. 2013; Raveendran *et al*. 2018
Catalases	Food preservation (with glucose oxidase), elimination of hydrogen peroxide from milk before cheese production. They are used as an indicator of the process of blanching in the processing of fruits and vegetables.	Raveendran *et al*. 2018
Cellulases	The cellulase enzymes are: endocellulase, exocellulase, and β-glucosidase. These enzymes have the important function of hydrolyzing cellulose with different purposes such as increasing the digestibility of fibers when they are used as food in ruminants, also their action favors the formation of fermentable sugars used for the production of ethanol, butanol and methane, as well as for the production of unicellular protein. In plants they act against diseases. Increase juice yields; They help clarify and stabilize wines. Restore the softness and color brightness of cotton. Contributes to the color stability of carotenoids. Improves the extraction of olive oil; helps to clean the fibers without damaging; eliminates rough protuberances on cotton fabrics; anti-redeposition of ink particles.	Kuhad *et al*. 2011; Tellez-Tellez *et al*. 2013
Cytochrome P450	In the fermentative process, they participate in the metabolization of phenanthrene to phenanthrene-trans-9,10-dihydrodiol and 2,2-diphenic acid as well as mineralizing it to CO_2, participate in the degradation of polycyclic aromatic hydrocarbons, carbamazepine and lignin. These enzymes have been associated with secondary oxidative metabolic pathways; they are used in the decoration of natural products that can be exploited in chemical production, through the biotransformation of fungal products; they are used in the production of pravastatin by the hydroxylation of mevastatin.	Bezalel *et al*. 1996; Golan-Rozen *et al*. 2011; Kelly and Kelly 2013
Lipases	Lipases are widely used in the detergent industry, in the food industry in the interesterification and transesterification of fats and oils, synthesis of precursors of the flavor of food, and maturation of cheeses. In the textile industry, it is applied to improve the dyeing capacity and promote the absorption of chemical compounds, such as antistatic, antiperspirant or antimicrobial. Feruloyl esterases have a potential role in the production of biofuels. Microbial lipases have a potential use in the degradation of ester-type xenobiotic compounds.	Dilokpimol *et al*. 2016; Piscitelli *et al*. 2017

Oxidoreductases	Within the main application of oxidoreductases enzymes is the lignin depolymerization that allows access to cellulose, for further degradation by cellulases for the production of biofuels, among other applications. For the production of compounds such as vanillin directly from the depolymerization of lignin; for the biotransformation of other compounds of high added value. Laccase enzymes are a group of blue copper-oxidases enzymes, which catalyze the oxidation of various substrates, such as polyphenols, aminophenols and aromatic amines, and since they are capable of degrading lignin, they can also degrade many highly recalcitrant environmental pollutants , they can also be used in the synthesis of pharmaceutical compounds such as anesthetics, anti-inflammatories, antibiotics, sedatives, etc., which include triazolo (benzo) cycloalkyl thiadiazines, vinblastine, mitomycin, dimerization of penicillin X, cephalosporins and dimerized vindoline; cosmetic and dermatological preparations containing oxidoreductases have been developed to lighten the skin, for deodorants, for personal hygiene products, including toothpaste, mouthwash, detergent, soap and diapers, which could have an effect on reducing allergies.	Upadhyay *et al*. 2016; Martínez *et al*. 2017; Naghdi *et al*. 2018
Pectinases	Functionally pectinases can be categorized as exo and endo polygalacturonases (which hydrolyse glycosidic α-(1-4) bonds), pectin esterases (which remove acetyl and methoxyl groups from pectin), pectin lyase and pectate lyase. The main application of the pectinases is in the food industry to increase the yields in the obtaining of juices and also in the clarification of these and of the wines, they are also applied in the wastewater treatment of fruit and vegetable industries, they are applied in the processing of textile fibers such as linen, jute and hemp; they are used in the fermentation of coffee and tea; its use helps in the extraction of vegetable oil and the treatment of paper pulp.	Tellez-Tellez *et al*. 2013
Proteases	Proteases are used to produce protein hydrolysates from casein, whey protein, and soy protein for the manufacture of dietetic and health products; in the production of cheeses; in the dehairing and bating of skins and hides; in the modification of food ingredients, for the development of flavors and as a meat tenderizer; in the pharmaceutical and cosmetic industry they are used in the elimination of the keratin, to eliminate scars, to regenerate the epithelia, they can hydrolyze the peptide bonds of the keratin, the collagen and the elastin of the skin. Ingredient of biological detergents, helps in the coagulation of the blood; treatment of inflammatory diseases.	Tellez-Tellez *et al*. 2013; Souza *et al*. 2015
Xylanases	Endoxylanases, exoxylanases, and β-xylosidases are the main xylanases. These enzymes are considered additives that are used to increase bread volume; they release pentoses in the enzymatic treatment of animal feed and participate in the degradation of agricultural waste, they are used in the bio-bleaching of pulp and paper. They are also ingredients in detergents that help the care of fabrics. They are thickeners and antifreeze additives, and they participate in the production of biofuels.	Tellez-Tellez *et al*. 2013

The degradation of lignin implies a high oxidation by class II peroxidase enzymes (POD) that act on the basis of conserved catalytic sites and bind to manganese (Mn), and are classified as manganese peroxidase (MnP), lignin peroxidase (LiP), versatile peroxidase (VP), phenoloxidases (laccases), aryl alcohol oxidases, superoxide dismutase, or glyoxal oxidases (Hammel and Cullen 2008). However, the production of multiple lignin-modifying isoenzymes has been reported for wood-degrading fungi, which are encoded by several structurally related and apparently redundant genes, and their expression depends on the culture conditions, presence of inducers, and other conditions (Salame *et al*. 2010).

Pleurotus ostreatus produces laccases and POD, such as MnP and VP, as the main ligninolytic activities. It has been suggested that VP is a hybrid of MnP-LiP, which oxidizes different substrates depending on the existence of Mn^{+2}, its crystalline structure, and theoretical molecular models (Martínez 2002). The absence of LiP in *P. ostreatus* has been confirmed by genome information (Knop *et al*. 2015). The ligninolytic system of *Pleurotus* spp. requires the continuous production of hydrogen peroxide (H_2O_2) for peroxidase enzyme activity (Gutiérrez *et al*. 1994). The H_2O_2 generating oxidases described in ligninolytic fungi are glyoxal oxidase and pyranose oxidase (Daniel *et al*. 1994), as well as aryl alcohol oxidase (AAO) (Hernández-Ortega *et al*. 2012). Additionally, two different peroxidases were identified in white-rot fungi: heme-thiolate peroxidases (HTP) and dye-decolorizing peroxidases (DyP). They showed differences in their amino acid sequence and their protein structures making them different from class II PODs, which justifies their classification as unique superfamilies (Hofrichter *et al*. 2010). Homologous proteins were identified as type DyP/DyP through analysis by PSI-BLAST. To date, only two reports on a DyP enzyme have been published for *P. ostreatus* (Faraco *et al*. 2007; Fernández-Fueyo *et al*. 2015). Certain DyP peroxidase types break down β-carotene and other carotenoids, as well as veratryl alcohol and dimeric non-phenolic β-O-4 type lignin model compounds (Liers *et al*. 2010). In contrast, the genes encoding HTP have been found in the genome of *Pleurotus*, but the corresponding enzymes have not been identified nor have they been isolated and characterized. Genetic study has been difficult because of the chromosomal arrangement that it presents (Ramírez *et al*. 2000), but with the advancement of molecular biology tools and the knowledge generated by classical genetics, the size and number of chromosomes of some species have been determined. However, there are differences depending on the methodology used. The genome size of *P. ostreatus* analyzed by an electrophoretic method has been reported to be between 20.8 Mb and 35.1 Mb and the number of chromosomes between 6 and 11. Using flow cytometry, the size of the genome ranges between 18.5 Mb and 28.7 Mb. In contrast, by microfluorometric measurements, the size is 24.0 Mb to 27.53 Mb. These differences are considered within the range of measurement error for plants or animals (Gregory *et al*. 2006).

The complete *P. ostreatus* genome (Table 2) contains 34.3 Mbp organized into 11 chromosomes (Larraya *et al.* 1999). These studies were supported by sequencing the genome of protoclones of *P. ostreatus* at the Joint Genome Institute (JGI). The sequencing of fungal genomes makes it possible to foresee the encoded proteins required for the biosynthesis of important metabolites (Sudhakar *et al*. 2018). In spite of the advances in comparative genomics, the genus *Pleurotus* is very much under-exploited and still has many potential applications in different areas of the industry that can be deciphered with their genomes. From this information, it is known that at least nine non-allelic genes encode for family members of the MnP gene. Manganese peroxidase oxidizes Mn^{+2} to Mn^{+3}. The first is a mandatory substrate for the enzyme that is necessary to complete the catalytic

cycle. In contrast, although VP preferably has a MnP activity and is structurally related to the gene and protein of MnP, it can oxidize aromatic substrates in the absence of Mn^{+2}. Manganese peroxidases from *P. ostreatus* belong to the subfamily called short MnPs. They can oxidize in the absence of Mn^{+2} to amines, phenols, and small dye molecules because they contain an additional active site that has an exposed heme edge (Morales *et al*. 2012). The peroxidases encoded by the genes mnp3, mnp6, mnp7, mnp8, and mnp9 are characterized as short MnP enzymes (MnP3, MnP6, MnP7, MnP8, and MnP9, respectively), while mnp1, mnp2, mnp4, and mnp5 code for VP (VP1, VP2, VP4, and VP5, respectively) (Salame *et al*. 2010). The high number of POD suggests a differential regulation, potential redundancy, and/or diversity in their properties. Additionally, four DyP genes, three HTP/CPO genes (chloroperoxidase), and four genes of glyoxal oxidase have been identified in the genome of *P. ostreatus* (Ruiz-Dueñas *et al*. 2011; Kersten and Cullen 2014). The family of cytochrome P450 also plays an important role in white-rot fungi (Ichinose 2013). It has been suggested that the members of this family are involved in the degradation of polycyclic aromatic hydrocarbons, carbamazepine, and lignin (Golan-Rozen *et al*. 2011).

Table 2. Genome Sizes of the *Pleurotus* spp.

Species	Size (Mbp)	Record	Method	Cell	Reference
P. pumonarius (Fr.) Quél.	36.56	REG s2	DAPI-PC	PC	Bresinsky *et al*. 1987
	30.0		CHEF	PC	Peberdy *et al*. 1993
	35.63	REG 4b	DAPI-PC	PC	Wittmann-Meixner 1989
	34.66	REG J3	DAPI-PC	PC	
	32.21	REG 6v	DAPI-PC	PC	
P. ostreatus (Jacq.) P. Kumm.	36.61	REG AM4	DAPI-PC	PC	
	35.82	SAMN05327826	WGS	PC	NCBI
	35.60	PC9v1.0	WGS	PC	JGI 2018
	35.30	PC9 Monocaryotic protoclone	CHEF	PC	Larraya *et al*. 1999.
	34.7	PC15 Monocaryotic protoclone	CHEF	PC	
	34. 30	PC15; SAMN02746102	WGS	PC	JGI 2018
	33.25	TAAM 142824	PI-FC	S-spore print	Kullman *et al*. 2005
	31.10		CHEF	PC	Peberdy *et al*. 1993
P. sapidus Syll. fung. (Abellini)	26.1			PC	
P. sajor-caju (Fr.) Singer	20.0			PC	

Species	Size (Mbp)	Record	Method	Cell	Reference
P. eryngii (Fr.) Singer	52.23	REG 3q	DAPI-PC	PC	Wittmann-Meixner 1989
	43.78	Strain 183	WGS	PC	NCBI
P. dryinus (Pers.) P. Kumm.	36.61	REG B1	DAPI-PC	PC	Wittmann-Meixner 1989
P. djamor Rumph. ex Fr.) Boedijn	66. 87	REG J5	DAPI-PC	PC	
P. djamor var. *roseus* Corner	65.90	Strain NBRC 31859	WGS	PC	NCBI
P. cystidiosus O.K. Mill.	21.97	REG 4Q	DAPI-PC	PC	Wittmann-Meixner 1989
P. calyptratus (Lindblad ex Fr.) Sacc	29.29	REG 9c	DAPI-PC	PC	
	37.11	TAAM 157761 (PU)	PI-FC	N-Intact nuclei	Kullman 2000

PI-FC - propidium iodide staining and flow cytometry; DAPI-PC - photometric cytometry stained with 4′,6-diamidino-2-phenylindole; CHEF - pulsed-field gel electrophoresis, contour clamped homogeneous electric field; WGS - whole genome sequencing; and PC - pure culture

Catalases from *P. ostreatus* are central components of enzymatic detoxification pathways. These enzymes can mitigate oxidative damage by catalyzing H_2O_2 in water and molecular oxygen (Kwok *et al*. 2004). Wang *et al*. (2017) analyzed two catalase genes (obtained from the genome) and found that the gene structures were variants between Po-cat1 and Po-cat2. The highest catalase activity was observed in the mycelium, followed by the primordium, fruiting bodies, and spores. Only one catalytic band (Po-cat2) was detected *via* native-polyacrylamide gel electrophoresis (PAGE). The catalase Po-cat2 plays a catalytic role in the decomposition of H_2O_2 during the life cycle of *P. ostreatus*, and was regulated 44 times (primordia), 70 times (fruiting bodies), and 323 times (spores) more than the mycelia. The expression of Po-cat1 was barely detectable in the mycelium and the maximum expression was in the spores. The results indicated that the two genes can play different roles during development. Activity in the spores may suggest that there are large differences in the catalases between fungi. Some can use the H_2O_2 detoxification pathways, glutathione peroxidase, and thioredoxin reductase systems, and can work in *P. ostreatus* spores (Sofo *et al*. 2015).

The fungus *P. ostreatus* can be considered a valuable source of oxidases, which is expected to be confirmed with the publication of the genome. However, the complex of these enzymes still needs to be clarified. It has been reported that *P. ostreatus* produces several laccases. They are the most studied enzymes because they have great potential for use in biotechnological processes. Hoegger *et al*. (2006) reported that the phylogeny of the laccase gene does not strictly follow the phylogeny of the species, and there are doubts about whether these enzymes evolved because of the lifestyle of the corresponding fungal species or an unknown factor. Kües and Ruhl (2011) stated that enzymes in fungi have

undergone separate evolutionary pathways by developing uncontaminated laccase activities (untainted) (*sensu stricto* laccases, although they may show some ferroxidase activity) (Rodríguez-Rincón *et al*. 2010). For *P. ostreatus*, 12 laccase genes were predicted (Lettera *et al*. 2010; Lundell *et al*. 2010). Six genes appear grouped in the subtelomere region of chromosome VI, and the others are assigned to chromosomes IV, VI, VII, VIII, and XI (two genes) (Pérez *et al*. 2009). Apparently, one chromosome codes for a laccase. Only 10 are grouped within subfamily 1 of laccases (LACC1, 3, 4, and 6 to 12). The laccase LACC2 (= POXA3) belongs to subfamily 2 of laccases (Kües and Ruhl 2011), which is a heterodimer with another small protein found in two differentially glycosylated enzymes (Giardina *et al*. 1999; Palmieri *et al*. 2003). Proteins of this type are rare in basidiomycetes and have only been reported in *P. ostreatus*, *Armillaria mellea*, and *A. bisporus* (Perry *et al*. 1993; Curir *et al*. 1997). Finally, LACC5 is included within the group of fungal ferroxidases (Kües and Ruhl 2011). This heterogeneity is due to the existence of several genes, but the isoenzyme pattern is even more complex as a result of post-translational modifications (proteolytic processing, glycosylation, *etc*.). Castanera *et al*. (2012) reported through a transcriptional analysis that the expression of laccase genes is positively regulated in induced submerged fermentation (aqueous extract of wheat straw), but negatively regulated in solid-state fermentation. The LACC10 and LACC2 genes were responsible for the activity of laccase in submerged fermentation after induction and additional activity not yet characterized (Unk1) was observed. It seemed that the expression of this isoenzyme was not induced by the wheat straw extract, which could have been because of the general conditions of the culture system, rather than the effect of the presence of phenolic inducers. Thus far, Unk1 has not been linked to any laccase gene. The POXC laccase enzyme (LACC10) is produced under different growth conditions (Palmieri *et al*. 1993). This protein (ID: 1089723) is negatively regulated when the fungus begins the fruiting process, so it has been suggested that it is important during vegetative growth (Pezzella *et al*. 2013). Despite the importance of these enzymes and the extensive work that is being generated around them, the transcriptional regulation of laccase gene expression is still poorly understood (Qi *et al*. 2017). However, it is known that the enzymatic activity of laccases and the transcription profiles of genes differ greatly between strains, culture systems, nutrients, environmental factors, *etc*.

Proteases are a complex group that catalyze hydrolytic reactions that produce peptides and amino acids from proteins. They have diverse properties and great importance in metabolism, biochemical functions in metabolic pathways, and cell signaling, as well as great importance in the pharmaceutical industry, food, and detergents (Kudryavtseva *et al*. 2008). It has been reported that a protease of a fruiting body from *P. eryngii* has a molecular weight of 11.5 kDa, according to sodium dodecyl sulfate-PAGE. The activity was negatively affected by pepstatin A, which indicated that it is an aspartic protease. It showed some inhibitory activity against HIV-1 reverse transcriptase (Wang and Ng 2001). It is important to mention that Gene Ontology (GO) is a project to develop and use ontologies to support the biologically meaningful annotation of genes and their products in a wide variety of organisms supported by organism databases and other bioinformatic resources (The Gene Ontology Consortium 2008). In the genome of *P. eryngii* (JGI 2018), up to six proteases can be found, which have been given different GO functions. An alkaline serine protease with a molecular mass of 28 kDa was isolated from the fruiting body of *P. citrinopileatus*. These enzymes may be important for its saprophyte mode of existence (Cui *et al*. 2007). In another study, an extracellular serine protease (of the subtilisin type) was isolated. This is a monomeric glycoprotein (75 kDa) that has an important function in the

regulation of laccase activity for *P. ostreatus* and modification and/or activation of different isoenzymes (Palmieri *et al*. 2001). *Pleurotus ostreatus* presents three proteases that coincide for some GO functions (JGI 2018).

For some enzymes, functional redundancy has been reported. This has also been reported to apply to genes that code for glycoside hydrolases and carbohydrate esterases, as well as to several genes of these enzymes. It was determined through omic studies that genes coding for these two enzyme types are positively regulated in wood-degrading fungi, including *P. ostreatus*, when they are grown on lignocellulosic substrates. Therefore, it has been suggested that they may have an important role in degradation. However, it may not directly degrade lignin (Nakazawa *et al*. 2016).

It has been reported that some basidiomycetes produce lipolytic enzymes (Lin and Ko 2005; Singh *et al*. 2014). To date, lipases have not been isolated in *P. ostreatus*. The PoceA1 gene encodes a putative CE1 protein (acetyl xylan esterase). Dark degradation fungi mostly lack the gene that codes for this protein, but it is present in white degradation fungi (Riley *et al*. 2014; Rytioja *et al*. 2014), although it does not seem to be directly involved in lignin degradation. After performing a homology search in the database (JGI 2018), it was found that *P. ostreatus* presents a possible CE1 protein. However, Nakazawa *et al*. (2016) stated that there could be another gene coding for CE1 in the genome of *P. ostreatus*, which can complement the function of Poce1A, or that this gene codes for feruloyl esterase. Meanwhile, Riley *et al*. (2014) reported the presence of two genes encoding CE1 in the genome of *P. ostreatus*. The lipolytic enzymes can hydrolyze or synthesize ester bonds, which depends on the amount of water present in the reaction (Glogauer *et al*. 2011). They are grouped into two main families, lipases and carboxylesterases.

Depending on the specificity of the substrate, it is possible to distinguish between lipases that hydrolyze triacylglycerols with chains of fatty acids of more than C10 and carboxylesterases that hydrolyze glyceroesters with chains of fatty acids that are C10 or shorter in length (Verger 1997). Piscitelli *et al*. (2017) conducted a search for lipolytic coding genes in the genome of *P. ostreatus* and found 53 possible lipase genes and 34 possible genes that code for carboxylesterase, with five genes in common between the groups. In *P. ostreatus* cultures with different conditions, four lipolytic enzymes were found: Pleo-Lip241, PleoLip369, PleoLip103, and PleoLip104. The first two were expressed in the yeast *Pichia pastoris* and the sequences encoding PleoLip241 (2327 bp in length) and PleoLip369 (2213 bp in length) were obtained, which showed a 52.6% identity.

PROMOTERS RESPONSE ELEMENTS OF THE ENZYME GENES

Promoters can activate or inhibit the transcription of a gene, so it is important to know each of the genes in the enzymes of industrial importance and to look for the ability to regulate and control their expression in wild and improved organisms. Through knowledge of transcriptional factors, the yield of obtaining metabolites of industrial interest can be increased. The possible polymerase binding sites have been determined through genomics and the transcriptional factors and types of promoters present can be known through comparative genomics because the success of the application of genetic engineering for improvement is mostly dependent upon expression control. Interesting transcriptional factors have been found in fungi, which could be an alternative that should be tested in said area.

It has been found that the genes encoding POD in *Pleurotus* have different response elements in the 5'-upstream sequences (Table 3), including possible metal response elements (MRE), heat shock elements (HSE), response elements of cAMP (CRE), and elements of xenobiotic response (XRE) (Asada *et al*. 1995; Fernández-Fueyo *et al*. 2014). In general, the laccase gene promoter regions contain TATA sequences, which are necessary to initiate transcription, and at least one CAAT motif that is important in the control of transcription. The position of the TATA and CAAT elements differs between fungal laccase genes (Ballance 1986). Both motifs may be related to the conserved regions within the genes (Okamoto *et al*. 2003). As was previously mentioned, induction can be selective in different genes because of several physiological and environmental factors, such as in the promoters of the laccase genes in *P. ostreatus* and *P. sajor-caju*, which present MREs (Soden and Dobson 2003; Piscitelli *et al*. 2011). However, the possible regulatory sequences reported are distributed differentially. Additionally, transcriptional factors can be found to counteract the shock by temperature (HSE) or stress response (antioxidant response element, ARE). The NGAAN sequence repeated in any orientation for HSE has been found between eukaryotes (Mager and De Kruijff 1995; Galhaup *et al*. 2002). Soden and Dobson (2003) reported several AREs with a consensus sequence TGACNNNGC that differs in a pair of bases, Sp1 (mammalian response element) and AP1 (ARE). The possible CreA site was found for the first time in *Aspergillus nidulans* as a homologue of *Saccharomyces cerevisiae*. It is present in the promoter region of *P. sajor-caju* laccase. The CreA represses the expression of cellulases and xylanases (Strauss *et al*. 1999; Suto and Tomita 2001). Meanwhile, ACE1 seems to be responsible for the regulation of copper in the synthesis of laccase because induction occurred in some species even in the absence of MRE sites in the promoter region of the laccase (Litvintseva and Henson 2002). Several MREs have been reported, but there have been no reports on direct transcriptional regulation. Qi *et al*. (2017) tested the effect of an MRE. They isolated ltf4 (clone with MRE-binding protein from the POXC gene) of *P. ostreatus via* a yeast hybrid method. The clone ltf4 interacted with cMRE2, which showed that the regulation of the POXC (LACC10) transcription was mediated in the presence of copper in *P. ostreatus*. It was suggested that the POXC gene can participate in vegetative growth, while its transcription is negatively regulated when the fruiting process begins. The transcription of ltf4 was relatively stable, but only under the stimulation of some environmental conditions because the expression level of ltf4 notably increased after the addition of copper.

The peroxidase promoter region sequence present in the genome of *P. ostreatus* (PC9) shows different response elements (Table 3), such as SP1, AP1, AP2, and GATA, with HSE, MRE, XRE, and CRE, which may be involved in the regulation of their expression given by the environmental conditions. The HSE are the elements found most (in seven of the nine promoters, absent in mnp1 and vp3). The mnp6, vp1, and vp3 have only one MRE, while vp2 contains two. Finally, XRE is only present in vp2 and mnp3 (Fernández-Fueyo *et al*. 2014).

The promoter region of the lipase genes (PleoLip241 and PleoLip369) has presented elements sensitive to fatty acids (CCTCGG). It has been found that several fungal genes encode proteins involved in fatty acids metabolism (Hynes *et al*. 2006); HSE (NGAAN) (Mager and De Kruijff 1995); sites of union to NIT2 (TATCT), which is a nitrogen regulator (regulates nitrogen repression) (Marzluf 1997); possible response elements PRE (motifs ATATC and TGGGT); response elements of cAMP (motifs CGTCA and TGACG); O_2-sitio (GATAA), which is the repression of O_2 and unsaturated fatty acids (Nakagawa *et al*. 2001); Cre-A union sites (GCGGGG); and elements sensitive to stress

(STRE and CCCCT). These genes have been shown to mediate a transcriptional response to various environmental and physiological conditions (Treger *et al*. 1998). Several possible response elements were identified, which were differentially distributed along the promoter sequences (Table 3). In PleoLip369, a large number of HSE and a fatty acid response element (FARE) were also identified (Piscitelli *et al*. 2017).

Efforts have been made to improve the efficiency of heterologous expression, which is why it is important to know the regulation of the promoter areas that are responsible for gene expression because it is an alternative for improvement through the substitution of binding sites. This could reduce activity by sites that improve performance, such as for *Trichoderma reesei*, by replacing three CREI (carbon catabolite repressor) binding sites.

For binding sites that increase transcription, the cellulase yields in the presence of glucose in a culture medium proved to be a highly effective improvement strategy (Zou *et al*. 2012). Knowing the transcription factors that encode the genes is important for future studies of metabolite production improvement.

Omic Studies on *Pleurotus*

Omic tools offer a holistic view of cellular molecules, tissues, and organisms. They mainly study the detection of genes (genomics), mRNA (transcriptomics), protein production (proteomics), and metabolites (metabolomics) under certain environmental conditions without bias or in a specific way. The integration of all of these tools is known as systems biology (Fig. 1). Through the use of transcriptomic and proteomic techniques, the specific functions of the vegetative mycelium and fruiting body can be described, while identifying the genes responsible for inducing or repressing certain metabolic pathways. The genes that respond to stress (water, temperature, oxygen, light, *etc*.) are responsible for specific cellular responses (initiation of primordial formation, fructification, pigmentation, *etc*.). At the industrial level, they are of great help to develop technologies for obtaining fruiting bodies with better organoleptic characteristics. The fruiting process of certain species that require stressors (*e.g.*, *P. pulmonarius*, which requires low temperature stress to initiate fruiting) can be carried out without needing a more specialized infrastructure. This information could be complemented by knowing the proteins expressed under certain environmental conditions, cell age, and the nature of the substrate on which the different *Pleurotus* spp. grow.

Through metabolomic studies, the metabolites involved in each cell process and different culture conditions could be known because the capacity of the genus *Pleurotus* in the production of secondary metabolites of importance in different industries (food, medicinal, environmental, *etc*.) has been widely documented. However, more still needs to be known about the genus *Pleurotus*. Such studies should be focused on differentiating the role of genes whose function is still unknown. Integration will have to be done through systems biology to make greater progress in understanding the metabolism, cell differentiation, production of enzymes and secondary metabolites during fruiting, and the response to biotic and abiotic factors. Omic tools have been widely used in the study of fungi from the genus *Pleurotus*, and the conclusions from some studies are shown in Table 4.

Table 3. Regions in the Gene Promoter of *Pleurotus* spp.

	Putative Response Elements																	
	Gene	TATA	CAAT	GC	HSE	NIT2	ARE	PRE	MRE	XRE	CRE	STRE	ACE1	Sp-1	AP1	AP2	O2-site	FARE
P. ostreatus	**Laccases[a]**																	
	lacc1	X	X (4)	X (2)			X		X									
	lacc2	X	X (5)	X					X (4)	X	X(2)	X						
	lacc3	X	X (2)				X		X (2)									
	lacc4		X (3)	X (2)			X (2)		X (5)									
	lacc6	X	X		X	X	X (2)		X (4)									
	lacc7	X	X (4)				X (3)		X (2)									
	lacc8	X (2)	X (3)				X		X (3)									
	lacc9	X	X	X (2)				X (2)	X (5)		X							
	lacc10	X	X (3)	X (3)	X	X (3)			X (4)	X								
	lacc11	X	X (2)	X	X	X			X		X	X						
	lacc12	X	X (2)						X (2)	X	X							
	Peroxidases[b]																	
	mnp1	X	X											X(2)				
	mnp2	X	X		X										X	X		
	mnp3	X	X		X									X				
	mnp4	X	X		X									X(2)				
	mnp5	X	X		X									X(2)				
	Mnp6	X	X		X				X					X				

	Gene	TATA	CAAT	GC	HSE	NIT2	ARE	PRE	MRE	XRE	CRE	STRE	ACE1	Sp-1	AP1	AP2	O2-site	FARE
	vp1	X	X		X				X		X			X(2)				
	vp2	X	X		X				X(2)	X				X(2)				
	Vp3	X	X						X					X				
	Lipases[c]																	
	PleoLip241	X	X	X	X	X(2)		X(3)	X		X	X(5)					X	X
	PleoLip369	X	X	X	X(20)	X(2)		X			X	X					X	X
	Catalase[d]																	
	Po-cat1				X													
	Po-cat2				X													
P. sajor-caju	**Laccases[e]**																	
	lacc1	X	X				X	X		X				X				
	lacc2	X	X (2)															
	lacc3	X (2)	X (2)			X		X			X					X		
	lacc4	X	X (4)		X	X	X (4)	X	X	X	X (2)		X	X (2)	X(2)	X		

[a]Piscitelli *et al*. (2011); [b]Fernández-Fueyo *et al*. (2014); [c]Piscitelli *et al*. (2017); [d]Wang *et al*. (2017); and [e]Soden and Dobson (2003)

Table 4. Advances in the Knowledge of the Biology of *Pleurotus* spp. through Omic Tools

Omic Tool	Analysis	Scientific Contribution	Reference
Genomics	Construction of genetic maps	Location and identification of the gene or group of genes and their possible function	Larraya *et al*. 2000
	Correlation of chromosomal regions with phenotypic characteristics	Selection and development of better strains by genetic markers	Larraya *et al*. 2003
	Genome sequencing	Genome comparison, gene function identification	JGI 2018
	Structural analysis of sequences	Knowledge of the evolutionary dynamics of *Pleurotus*	Castanera *et al*. 2016
	Comparative analysis of transposable elements	Determination of patterns in the complete genomes of *P. ostreatus*	Castanera *et al*. 2016
Transcriptomics	Gene expression of hydrophobins: POH1, POH2, and POH3	Determination of its association with the growth rate and availability of nutrients	Peñas *et al*. 2002
	Detection of transcripts	Attribution of specific activities to some predicted genes or ORF, as is the case for some laccases in *P. ostreatus*	Castanera *et al*. 2012
	Specific activities of aegerolisines in *P. eryngii*	Analyses revealed that Pe.PlyA and Pe.ostreolysin from *P. eryngii* are functional orthologs from PlyA and ostreolysin from *P. ostreatus*.	Kurahashi *et al*. 2014
	cDNA libraries of different growth stages of *P. ostreatus*	Genes associated with primordia formation were identified: Hydrophobins (POH2 and POH3) and metallothioneins and differentiation of fruiting bodies: Hydrophobins (POH1 and vmh1).	Joh *et al*. 2007
	Gene expression at different stages of development of *P. ostreatus*	Identification of mitochondrial proteins and of respiratory processes, such as cytochrome P450, enzymes for carbon metabolism (synthesis of chitin and metabolism of carbohydrate and lipids), genes that code for stress-associated proteins, such as Hsp12, histidine kinases, PriA, and metalloproteases (reported as growth promoters).	Lee *et al*. 2002; Lee *et al*. 2009
	Gene expression in the lamellae of *P. ostreatus*	Enzymes associated with central metabolism (pyruvate dehydrogenase, glucose dehydrogenase, endopeptidase, cellobiohydrolase, as well as permeases) and specific conidiation proteins	Park *et al*. 2006
Proteomics	Proteomics on the stimulating effect of Tween 80 on mycelial growth of *P. tuber-regium*	Positive regulation of heat shock proteins was reported, which could help maintain cell viability under environmental stress.	Zhang *et al*. 2012

	Mycelial proteomics of *P. ferulae* caused in the presence of extracts of *Ferula songorica*	Proteins were identified, including one of heat shock (monooxygenase containing flavin and one NADPH: quinone oxidoreductase type IV). The change in expression of these proteins confirmed that the extracts remarkably affected the growth and primary and secondary metabolism of *P. ferulae*.	Bai *et al*. 2014
	Integrated secretome analysis (tool for mushrooms)	Identification of specific sequences, such as the signal peptide: SECRETOOL platform, designed to identify by sequence those proteins capable of being secreted	Cortázar *et al*. 2014
	Difference of *P. ostreatus* in the presence of selenium	17 positively expressed proteins and 34 negatively expressed proteins were detected; proteins involved in the metabolism of substances and energy in the cell were identified.	Wang *et al*. 2015
	Microsome proteome of *P. ostreatus*	Enzymes, such as epoxide hydrolase, involved in the degradation of xenobiotics, were identified; the microsomal proteins identified were part of the mitochondrial energy metabolism.	Petráčková *et al*. 2016
	Secretoma of *P. ostreatus* on lignocellulosic material	It was found that 31% to 39% corresponded to oxidoreductases (laccases, VP, and MnP), and 14% to 16% to CAZy.	Fernández-Fueyo *et al*. 2016
Metabolome	Metabolomic analysis of industrial fermentation process of pleuromutilin of *P. mutilus*	It was determined that the highest level of intermediates of the TCA cycle and amino acids was during the propagation of the inoculum and higher metabolism of carbon and nitrogen in *P. mutilus* was found before inoculation.	Yang *et al*. 2012
	Metabolomics to identify biomarkers of *Laetiporus sulphureus*	The metabolome of *L. sulphureus* was compared with that of seven edible fungi, including *P. ostreatus*, to identify biomarkers that could be related to the production of metabolites of industrial interest.	Woldegiorgis *et al*. 2015
	Metabolic analysis of the interspecific interactions between white-rot fungi	The changes to the metabolites were determined *in vivo* during the competitive interaction between *Trametes versicolor*, *P. ostreatus*, and *Dichomitus squalens*; differential metabolites were identified in the interaction zone; many metabolites in the metabolic pathways of carnitine, lipids, ethylene, and trehalose were remarkably positively regulated, and are involved in the defensive response to abiotic and/or biotic stressors.	Luo *et al*. 2017

Liu *et al*. (2017) performed proteome and transcriptome studies on a dikaryotic strain (DK13×3) generated from two monokaryotic strains (MK13 and MK3) of *P. ostreatus*. The transcripts of the three strains were more than 10000, but the expression profile in DK13×3 was similar to that in MK13. It was observed that in the DK13×3 strain, the genes involved in the use of macromolecules, synthesis of cellular materials, resistance to stress, and signal transduction were more regulated than the strains that gave rise to it.

The possible forms of gene differential expression that may contribute to heterosis include variation in the presence/absence of: A) genes expressed in both parents, but not in hybrids; B) genes expressed in one parent and hybrid, but not in another parent; C) genes expressed in one parent, but not in another parent or hybrid; and D) genes expressed only in a hybrid, but not in both parents. The gene expression for additivity/non-additivity occurs according to A, which has been reported in other studies (Castanera *et al*. 2013). Through this work, evidence was provided about the growth advantage of a dikaryon organism over a monokaryon, so that in the improvement of the characteristics of the strains (resistance to the environment), it will be possible to select monokaryon organisms and make the crosslink. Therefore, these improvements can be observed in the formation of the dikaryon (Liu *et al*. 2017).

In another study, from information at the molecular level generated by the sequencing of the genomes of two strains of *P. ostreatus* (PC15 and PC9) and one strain of *P. eryngii* (ATCC 90797) (JGI 2018), and other computer technologies, KEGG pathway maps were generated that classified proteins and genes involved in specific metabolic pathways, which helped to deduce their physiological role in the organism. The KEGG database classifies a gene by the function of the biological system, such as a cell, organism, and the ecosystem, using information from the molecular level (large-scale molecular data sets generated by genome sequencing). Then, bioinformatic programs are used to relate the differentially expressed genes (DEG) in the KEGG pathways. The KEGG numbers reported for *P. ostreatus* (for both PC15 and PC9) and *P. eryngii* are similar. In all three cases, KEGG was not reported for "Replication and Repair" and "Sorting and Degradation" (Table 5). These tools can give an idea of the possible functions of the studied metabolites.

Zhong *et al*. (2017) reported the changes in *P. ostreatus* gene expression of the interaction mentioned by Luo *et al*. (2017). The gene expression was 1730 DEG for *Dichomitus squalens* (1056 upregulated and 674 downregulated) and 1507 DEG for *Trametes versicolor* (911 upregulated and 596 downregulated). Both antagonistic relationships presented 638 upregulated genes and 422 downregulated genes, which implied a common antagonism mechanism in both interaction zones. The 18 possible functional groups of DEGs found within the positively regulated functional groups were responsible for the response to oxidative stress, such as aldo/keto reductase, glutathione-S-transferase, cytochrome P450, laccase, NAD(P) binding proteins, alcohol oxidase, and oxidoreductase, as well as enzymes involved in the metabolism of carbohydrates (CAZy), including carbohydrate esterase, glycoside hydrolase, and cellulose-binding protein, which are all involved in carbohydrate metabolism. Additionally, the functional groups were responsible for the enzymes involved in the tricarboxylic acid (TCA) cycle (citrate synthase, malate dehydrogenase, succinate dehydrogenase, and succinyl-CoA synthetase). They also performed a GO function analysis of the DEGs, and 31 functional classes were obtained in two GO categories. For the category of molecular function, the three main ones were oxidoreductase, catalytic, and hydrolase activities acting on glycosyl bonds. Within the category of biological function were those of the carbohydrate metabolic process and oxidation-reduction process, which are reported in the genome of *Pleurotus* spp. (Table 5).

When performing the DEG analysis to determine the KEGG pathway, the main processes were amino acid metabolism (tyrosine metabolism pathway), two xenobiotic metabolic pathways (metabolism of xenobiotics by cytochrome P450 and drug metabolism, and cytochrome P450), carbohydrate metabolism, fatty acid biosynthesis, and isoquinoline alkaloid biosynthesis, which have been reported for the *Pleurotus* genome (Table 5). The information obtained through the GO and KEGG analyses provided functional information, and the main DEG metabolic pathways were involved in the antagonistic interaction. Many of the results match the metabolomic study by Luo *et al*. (2017).

Table 5. Molecular Function and Role in the Biological Processes of the Gene Products of *P. ostreatus* and *P. eryngii*

	P. ostreatus PC15	*P. ostreatus* PC9	*P. eryngii* ATCC 90797
Genome Size (Mb)	34.3	35.6	
Genes	12330	12206	
Transposable Element	9.95	4.9	
KEGG Metabolic Pathways			
Amino Acid Metabolism	535	531	524
Biosynthesis of Polyketides and Nonribosomal Peptides	108	122	125
Biosynthesis of Secondary Metabolites	323	311	299
Carbohydrate Metabolism	587	556	610
Energy Metabolism	131	127	129
Glycan Biosynthesis and Metabolism	240	216	246
Lipid Metabolism	508	474	473
Metabolism of Cofactors and Vitamins	417	388	396
Metabolism of Other Amino Acids	119	110	126
Nucleotide Metabolism	289	262	327
Overview	358	346	352
Xenobiotics Biodegradation and Metabolism	358	344	341
KEGG Regulatory Pathways			
Replication and Repair	0	0	0
Sorting and Degradation	0	0	0
Transcription	235	221	242
Translation	60	51	64
GO Functional-enrichment			
Biological Process	3418	3701	3734
Cellular Component	1938	2003	2089
Molecular Function	5215	5400	5588
Obsolete Biological Process	325	341	335
Obsolete Cellular Component	15	12	1
Obsolete Molecular Function	200	202	234
Reference	JGI 2018		

Li *et al*. (2018) analyzed the transcriptional response of *P. eryngii* in the presence of cadmium chloride ($CdCl_2$) and nitric oxide (NO). The DEG obtained from the mycelium grown with 50 μM $CdCl_2$ was enriched in cellular components and molecular function, which suggested that they notably influenced the response of *P. eryngii* to heavy metals. The GO analysis of the functional enrichment showed that numerous unigenes were downregulated and involved in hydrolase activity and the binding of iron ions. This

suggested that hydrolases and iron ion binding activities were inhibited in mycelia exposed to $CdCl_2$. The GO categories of oxidoreductase activity in the oxidation-reduction and lipid biosynthetic process were overrepresented in the upregulated unigenes of mycelia that were exposed, which indicated that these genes were modified to adapt to heavy metal-induced oxidative damage. The KEGG analysis showed that several DEGs involved in lipid metabolism and the synthesis of secondary metabolites were positively regulated in exposed mycelia. Additionally, the unigenes of glycerolipids and secondary metabolites, such as steroids, glycerolipids, and terpenoids, were positively regulated under heavy metal stress conditions. The KEGG analysis also showed that the inhibition of secondary metabolite synthesis coincided with an increase in the energy rate and carbohydrate metabolism. When NO was added to cultures containing extremely high levels of heavy metals, it initially crossed the cell wall of the hyphae by diffusion. It then activated the process of oxidation-reduction of the hyphae, transcription factors, and increased the metabolism energy and carbohydrates, as well as increased the biosynthesis or metabolism of coenzymes and cofactors, which finally improved the tolerance of *P. eryngii* to heavy metals. This study illustrated the mechanisms by which NO enhances fungal responses to environmental stresses at the level of gene expression, thus laying the groundwork for future studies on the presence of NO in other fungal species.

Proteomics has become a tool of great importance to understand the role of proteins based on their participation in biological processes. Xiao *et al*. (2017) reported the proteomic profile of *P. ostreatus* grown with different carbon sources. The enzymes related to the oxidative stress response (superoxide dismutase, ascorbate peroxidases, and glutathione reductase) increased when *P. ostreatus* was grown in the presence of lignin, because the response of the mycelium to H_2O_2 was promoted. Most of the enzymes that participate in energy metabolism were present in smaller quantities in the presence of lignin. When it was grown in the presence of xylan and carboxymethylcellulose, the enzymes involved in the transformation of sugars through different metabolic pathways increased, which was reflected in better growth (biomass). It has been reported that lignin in agroindustrial residues limits mycelial growth due to the complex structure of the substrate and difficulty of using polysaccharides. It is of great importance to know the lignocellulose-fungi interactions to understand the ecology of these organisms and optimize the bioconversion of said substrates into products of biotechnological importance. This can be done by integrating the information obtained through different techniques.

Zhang *et al*. (2012) performed a proteomic analysis of *P. tuber-regium* to determine the effect of stimulating agents (Tween 80) in a liquid culture. They observed that there was a positive regulation on heat shock proteins that help to maintain cell viability under conditions of stress and on ATP-citrate lyase (two isoforms) that could inhibit the activity of the TCA cycle and consequently promote exopolysaccharide production. Zhang and Cheung (2011) reported that as the growth of *P. tuber-regium* progressed (old mycelium) in a submerged culture, environmental stress also increased, which caused an increase in the number of proteins and damaged cellular components. Therefore, by favoring the positive regulation of heat shock proteins, they reduced the environmental tension in the mycelial cells, which helped to maintain their structure and prevent disintegration. It has been reported that the HSP70 protein (thermal shock) intervened in different protein folding processes in almost all of the cellular compartments, can prevent aggregation, facilitate the proteolytic degradation of unstable proteins, and is responsible for the assembly of peptides that were imported into the mitochondria and the export of certain secretory proteins (Plesofsky-Vig and Brambl 1995; Bakau and Horwich 1998). However,

because the genome of *P. ostreatus* contains 153 members of this gene family (Zhang *et al*. 2018), more studies are required to understand the specific function of these enzymes.

The process of wood decomposition mediated by fungi has an important role in the process of storage and release of nutrients in forest ecosystems (Delaney *et al*. 1998). In addition, a substrate is colonized within natural systems by different organisms, which causes a confrontation between species of the same kingdom. When different fungi species are confronted, the direct combative interactions between mycelia to defend or compete for resources are always accompanied by changes in the mycelial morphology and secretion of extracellular metabolites, among which are enzymes (oxidative), alcohols, aldehydes, ketones, terpenes, and aromatic compounds, most of which are important in the defensive response to interspecific antagonism in the zones of interaction (Gregorio *et al*. 2006; Evans *et al*. 2008). Some studies have shown that metabolites secreted in the zone of interaction between two fungi species caused some morphological changes in the mycelium and there was a response by the metabolites against the stress (antagonistic interactions) (Bertrand *et al*. 2013). The mechanism of metabolic regulation against the condition is not yet clear. It is known that each species can activate various ways to improve the competitive potential, which could mainly involve signal molecules, growth inhibitors or toxins, and their by-products in the interaction zones.

Luo *et al*. (2017) studied the metabolomics of three mycelia (*T. versicolor*, *D. squalens*, and *P. ostreatus*) that interacted with each other and formed a band of a light-yellow pigment in the zones of interaction, which could have been caused by an antagonism between fungal species. A total of 279 metabolites were detected, which generally increased their regulation in the interactions. The detected intracellular metabolites participated in the following pathways: amino sugar and nucleotide, aspartate family, glutamate family, branched chain amino acids, carnitine, glycerolipids, free fatty acids, phospholipids, nicotinate and nicotinamide, pyrimidine, purine, serine family (phophoglycerate derived), glucose, fructose, sucrose, and TCA cycle. With the exception of nicotinate and nicotinamide metabolisms, all of the other induced pathways belong to carbohydrate, amino acid, and lipid pathways, which indicated that a wide range of metabolic reactions of energy production were activated to compete because almost half of the compounds exhibited synergistic effects and were higher or lower in the interaction zones than in the individual strains. It was found that 16 metabolites increased and only one compound decreased in all of the interaction zones. Tryptophan was one of the compounds that was induced in the three interactions. Its production was increased 119.94 times in the interaction between *T. versicolor*-*P. ostreatus* and 71.73 times for *P. ostreatus*-*D. squalens* compared with the *P. ostreatus* isolate. Trehalose decreased in the three zones of interaction, probably because the fungus consumed its own trehalose or hydrolyzed glucose from trehalose to supply energy and carbon when growth and nutrient limitation occurred under stressful conditions. The changes observed during the interaction affected the synthesis of the cell wall, production of osmolytes, and regulation of carbon and energy in the zones of interaction. Because different antagonistic responses were triggered to compete for nutrients during combative interaction, it has been documented in several papers that laccase activity increases in response to stressful conditions (Díaz *et al*. 2011, 2013) and it has been suggested that the interaction between tryptophan and copper could induce the production of laccase (Diwaniyan *et al*. 2012).

Secretome is the set of proteins that presents a signal peptide and are processed in the endoplasmic reticulum and Golgi apparatus before their secretion (Lippincott-Schwartz *et al*. 2000). Zorn *et al*. (2005) analyzed the secretome of *P. sapidus* grown in a liquid

culture using peanut shells (*Arachis hypogaea*) or glass wool as a support material with glucose as the carbon source. The protein concentration was higher in the peanut shell cultures (270 against 170 mg/mL on the 7th d of culture), which indicated an enzymatic inducing effect from peanut shells. Polygalacturonase and peptidolytic (several metallopeptidases and serine-proteases) enzymes were detected, while the MnP and VP family were predominant in the cultures grown with glass wool. In contrast, the secretome analysis of *P. eryngii* reported by Xie *et al*. (2016) was conducted to look for the enzymes involved in lignocellulose degradation. This type of analysis provides details about the composition of extracellular enzymes. In the secretome of *P. eryngii* grown on a lignocellulosic substrate (ramie stalk), 87 non-redundant proteins were identified, 43 proteins had signal peptides, 79 proteins had no transmembrane helix, six proteins had a transmembrane helix (not expected to be secreted), and the following compounds were found: laccases, MnP3, MnP5, Mn-dependent peroxidase, aryl alcohol-oxidase precursor, alcohol-oxidase isoenzymes, glutathione-disulfide-reductase, phenol-oxidase, heme peroxidase (24% lignin-degrading enzymes), glycoside hydrolase family 7 cellobiohydrolase (GH), cellulose 1,4-β-cellobiose (33% cellulases), hemicellulases and pectinases (6.9%), proteases (elastinolytic metalloproteinase and aspartic protease), peptidases (astacin peptidase m12a, leucine aminopeptidase, and carboxypeptidase s1) (23% proteases and peptidases), and others (12.6%).

Monokaryotic *P. ostreatus* (PC9) was grown using poplar wood (woody-type substrate) or wheat straw (non-woody-type substrate) as a unique source of C/N and the secreted proteins were analyzed, as well as those from the glucose medium. More than 500 proteins were identified, where 34% in the straw culture, 15% in the glucose culture, and 6% in the poplar culture were identified as unique proteins. In the three growing conditions, a 20% increase was observed and the two lignocellulose cultures had a 19% increase. The different proteins were functionally classified as follows: (i) oxidoreductases, including LME; (ii) active in carbohydrates (CAZys); (iii) esterases; (iv) proteases; (v) phosphatases; (vi) other functional proteins; and (vii) proteins of unknown function. The semiquantitative analysis revealed that oxidoreductases were the main enzymes in both poplar (39% of total abundance) and straw (31%), while CAZys were produced in smaller quantities (14% to 16%). The LACC10 laccases were the main proteins in the two lignocellulosic substrates (10% to 14%) and LACC2, LACC6, LACC10, MnP3, and VP1 were also overproduced in large quantities in the lignocellulosic cultures. Among the 50 major proteins were seven CAZys, and only the CE16 acetylesterase was overproduced in the lignocellulose. When comparing the woody and non-woody substrates, β-glycosidase GH1 was more abundant in the poplar and GH3 was more prevalent in the straw. The versatile peroxidase VP2 was overproduced in the straw, while VP3 was only observed in the poplar. The lignocellulosic materials were analyzed by two-dimensional nuclear magnetic resonance. The analysis showed preferential elimination of lignin (determined from the aromatic signals) with respect to the polysaccharides (determined from the anomeric carbon signals). The fungus more easily degraded the syringyl units, according to their higher degree of methoxylation (which reduces their redox potential). The resinols that were also lignin substructures were degraded by the fungal treatment. The modification of lignin has been reported with and without the addition of mediators for VP (Fernández-Fueyo *et al*. 2014) and laccases; the appearance of reducing sugars in the wood and wheat straw suggested the partial depolymerization of polysaccharides by CAZys (Fernández-Fueyo *et al*. 2016).

Large-scale analysis has reported that fungi excrete small proteins, called small secreted proteins (SSP) that contain a signal peptide and a sequence of less than 300 amino

acids. Its function is not yet known, but it has been suggested that they participate in pathogenicity, symbiosis, and degradation (Alfaro *et al*. 2014). Small secreted proteins have also been reported in saprophytic fungi and some of them are similar to effector proteins of pathogenic fungi, which suggests that these proteins have alternate functions (Kim *et al*. 2016). The SSP and enzymes that catalyze degradation of lignocellulose are components of the secretome of *P. ostreatus*. Three genes were identified (ssp1, 2, and 3) by comparing the results with the JGI genome data of *P. ostreatus* PC9. In a recent study, when exposing *P. ostreatus* to aryl-alcohols, the compounds SSP, aryl alcohol dehydrogenases (AAD), and AAO were produced. The SSPs could act at the protein level (either by preventing transcription or protein degradation) or interact with metabolites involved in signaling or with other components because it was found that SSPs improve the production of the enzymes AAO, AAD, and VP. Therefore, it was suggested that SSPs can act as regulators, at least in some members of the ligninolytic system (Feldman *et al*. 2018).

It has been reported that apart from the enzymatic activity of the ligninolytic system, white-rot fungi produce other enzymes, which have been poorly investigated. Nakazawa *et al*. (2017b) identified two genes (chd1 and wtr1) in *P. ostreatus*, which caused the Mn^{+2}-dependent peroxidase activity to be almost completely lost. It was suggested that the chd1 gene encodes a chromatin modifier and wtr1 encodes a specific agaricomycete protein with a DNA binding domain. The chd1-1 mutation and the directed disruption of wtr1 hindered the ability of *P. ostreatus* to degrade lignin. The effect on the expression of certain MnP/VP genes was determined and suggested a complex mechanism where other factors are involved. The protein Pex1 is conserved between eukaryotes and is important in the biogenesis of peroxisomes. It was reported that the interruption of Pex1 showed defects in the growth of *P. ostreatus* hyphae in sawdust and certain minimal culture media because it causes a noticeable loss in the ability to biodegrade wood lignin, but not polysaccharides. This was attributed to defects in the glyoxylate cycle, which usually occur in peroxisomes, because it has been reported that this metabolic pathway is important in the degradation of lignin. It is important because it provides the H_2O_2 necessary for peroxidase activity or an alternation in the transcriptional regulation caused by the interruption of Po.pex1, which results in peroxisome malfunction. However, studies are still needed to specifically clarify the process of the factors involved in lignin degradation (Nakazawa *et al*. 2017a). It is important to highlight that these results were achieved by combining classical genetic techniques with sequencing technology (omic tools). This research must be complemented with all of the tools that are available to obtain important advances in phenotypic analysis and comparative transcriptomic/epigenomic analysis, to increase the understanding of the activity mechanisms and implication of diversity as well as processes that take place during lignin degradation. This is of great importance considering that lignocellulosic raw material is abundant. There are approximately 1300 million tons of agricultural waste in the whole world on an annual basis (Sarkar *et al*. 2012). It is an inexhaustible source for the production of chemical compounds and other value-added products from biological waste (Rogers *et al*. 2017).

Transposable elements (TEs) are a prolific source of biochemically active and tightly regulated non-coding elements, such as transcription factor binding sites and non-coding RNAs. Depending on the nature of their transposition intermediaries, TEs can be classified as class I (RNA) or class II (DNA). Class I elements are the most frequent TE in fungi (Amselem *et al*. 2015). The elements of both classes interrupt the colinearity of the genome between strains, produce polymorphisms of the strain, and silence genes in their

intermediations. Additionally, they block the transcription of genes where they are inserted. For Agaricomycotina, class I elements are dominant, while class II elements are restricted and generally do not comprise more than 1% of the genome (Castanera *et al*. 2017). The presence of helitrons has been reported in fungi as TEs in several sequenced genomes of ascomycetes and basidiomycetes (Labbé *et al*. 2012; Castanera *et al*. 2014). In *P. ostreatus*, two families of helitrons, HELPO1 and HELPO2, were identified and characterized, which showed differential expression patterns and distribution (Eugenio and Anderson 1968). Additionally, through the genome information of the *P. ostreatus* strains PC9 and PC15, it was possible to determine notable genomic differences in the regions corresponding to TEs, where 80 identified TE families represented 2.5% and 6.2% of the genome sizes for PC9 and PC15, respectively. Transposable elements are an important fraction of the genome of eukaryotes and prokaryotes. Fragments of DNA are able to mobilize in the genome of the host, which contributes to chromosomal rearrangement and transcriptional modulation.

ANALYSIS OF CELLULAR PROCESSES THROUGH OMIC TOOLS

Xie *et al*. (2018) reported the transcriptome of the fruiting body of *P. eryngii* during its development under blue light. The process of primordia and fruiting body formation, differentiated during the life cycle, is mediated by cellular processes and genetic, physiological, and environmental factors, within the latter of which is light. It has been reported to have an effect during the differentiation of the fruiting body and that blue light could induce differentiation and development of the fruiting bodies in *Hypsizygus marmoreus* (Jang *et al*. 2013), *P. ostreatus*, and *Coprinus cinereus* (Terashima *et al*. 2005). A total of 539 unigenes were identified as DEG, with 485 unigenes regulated by increments and unigenes regulated by a decrease in those exposed to blue light compared with the control group (which remained in darkness). Several genes play an important role in response to blue light (positively regulated), such as the white collar-2 type of the transcription factor gene (which was identified as a blue light receptor), a mating-type protein gene, and the MAP kinase gene. Three class I hydrophobin genes and pheromone receptors mediate the interactions between the fungus and the environment. Additionally, a series of genes in the mitochondrial respiratory chain (cytochrome b subunit of succinate dehydrogenase, cytochrome c1, NADH-ubiquinone oxidoreductase, and ubiquinol cytochrome-c reductase) were found to be positively regulated, which suggested that oxidative phosphorylation can participate in the response to blue light. Interestingly, the genes associated with carbon metabolism also regulated the amino acids and fatty acids. A KEGG analysis was performed. The DEGs were highly concentrated in the carbon metabolism pathways (glycosylation/glycogenesis) and amino acid biosynthesis, which suggested that the main blue light response pathways were those of primary metabolism during the formation of the primordium of *P. eryngii* for the formation of the fruiting body (Xie *et al*. 2018).

Fu *et al*. (2017) used RNA-sequencing technology for a comparative analysis of the transcription of *P. tuoliensis* (Bailinggu) in four stages of development: undifferentiated mycelium (Stage I), cold stimulation of mycelia (Stage II), primordia (Stage III), and fruiting body (Stage IV). The greatest number of DEGs occurred during the transition stage from vegetative to reproductive mycelium (II to III), which suggested that this period is the most active, and therefore, key to the development of *P. tuoliensis*. Some transcription factors were identified as MADS, C2H2, and FST4 (which have a role in the formation of

fungi and response to cold stress). In *Arabidopsis thaliana*, MADS is essential for fertilization to occur at the time of maximum reproductive potential (Moon *et al*. 2003). In the study by Moon *et al*. (2003), MADS maintained regulated expression during the last three stages of development, which indicated that it may be essential for morphogenesis. Ohm *et al*. (2010) found that FST4 in *Schizophyllum commune* is positively regulated during the development of the fruiting body and there was no fructification when FST4 was inactivated in a dikaryon. Using the KEGG pathways, it was determined that the cells use TCA to a greater extent during the stages of reproductive growth compared with the stages of vegetative growth (more energy is needed during reproductive growth) and has a crucial role in the supply of energy and carbon for growth, proliferation, and cellular development of *P. tuoliensis*. In addition, the main genes encoding the glycolytic enzymes in the pathway of glycolysis were positively regulated in the development of the fruiting body compared with the three other development stages. The unigenes related to MAPK signaling pathways dependent upon pheromones, starvation, and osmolarity were differentially expressed during the four stages, so they could be involved in cell growth and differentiation, development of mycelia, morphology of the fruiting body, and adaptation to cold stress stimuli.

In the culture cycle, blue light is also essential for the transition from the vegetative phase to the fruiting phase and the growth of the fruiting body. Although the undifferentiated mycelium stage can pass without light, the fructification stages need blue light. Those involved were the genes that code for white collar-1, cryptochrome, and photolyase. The first two are photoreceptors of blue light and the families of cryptochrome and photolyase with the ability to bind at flavin adenine dinucleotide and absorb blue light to perform different tasks. Therefore, they could play a key role in photomorphogenesis. However, this process is complex and requires more study. With the wide use of omics-based technologies, it has been possible to build genome-scale models for non-model organisms that can facilitate the choice of microbial pairings (Tagu *et al*. 2014). This could allow researchers to quickly generate hypotheses about metabolic linkages, which could be used to promote stable consortia between biomass degradation.

CONCLUSIONS

The fungi from the genus *Pleurotus* have been used to study the production of enzymes and different secondary metabolites because it is an edible fungus. Its components do not present health risks. Additionally, because its production is relatively simple with a short time for fruiting body formation, it has been used as a model to understand different processes of morphogenesis. The information obtained through genome sequencing and the use of omic tools has been of great help. Different comparative genomic analyses are being performed to better understand the genetic and evolutionary basis of several important cellular functions in the different industrial and environmental areas. This knowledge can identify bottlenecks in the production of enzymes and secondary metabolites and therefore increase yields and reduce production costs for application in different processes. In that sense, more studies are needed to learn more about the biological and genetic processes of the *Pleurotus* genus.

Research relating to edible mushrooms should be increased, mainly from the genus *Pleruotus*, to integrate the information generated by genomics, proteomics, transcriptomics, and metabolomics. Genomics will continue to emphasize functional

analysis and promote a systematic and integrated approach for biological, ecological, and genetic studies. The research must adopt a perspective of understanding by integrating the different information to obtain results and perceptions, which would not be possible using only traditional methodologies. One of the advantages of the omic sciences is that organisms are no longer analyzed in isolation, but as an integral component of a system to understand the functional biology. The vast majority of fungi are still remarkably under-exploited and the potential for production and utilization of metabolites in different areas of industry and the environment still needs to be deciphered.

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