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REVIEW ARTICLE

Mushrooms as Efficient Enzymatic Machinery

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ABSTRACT

Mushrooms are generous source of nutritional, medicinal compounds, and industrial uses of the mushrooms still need to be worked out for their important applications. Mushrooms have significant enzymatic machinery allowing their application in different industries. Capabilities of different members of mushrooms have encouraged researchers to investigate further applications of these macrofungi in fields other than food and pharmaceutical industries. Specially, owing to the current shortage in global resources, contamination caused by plastic components and the incredible increase in population worldwide needs alternatives through macrofungi. Therefore this review aims to put light spot on enzymes production by mushrooms.

INTRODUCTION

The fungal kingdom is full of members that have significant roles in different aspects, and improved life of humanity starting from penicillin that saved the life of millions, besides their applications in food, industry, and pharmaceutical fields. Mushrooms as higher Basidiomycetes and Ascomycetes contain secondary metabolites in their fruit bodies, cultured mycelium, and cultured broth [1-5]. Mushrooms are those macrofungi known from centuries as food and in traditional medicine. Science has proven the contribution of mushrooms as nutritional foods and as efficient component in medical and pharmaceutical fields [6-10]. This is due to their richness in biologically active compounds that belong to different chemical classes such as peptides, terpenes, phenols, fatty acids, glucans, flavonoids, and others. There is an elevated scientific interest in mushrooms due to the high number of identified mushroom species (over 14,000 species and counting) [11-15]. Also, due to the achieved advances in analyses techniques and instruments that allow accurate analysis and estimation of components originated from each mushroom species. Especially that even within the same species, a considerable difference in metabolic profile and chemical composition is noticed. Many literature have described the biological activities of components and extracts originated from mushrooms [16-20].

Mushrooms have been used in many sides of human activity for many years. Some of these mushrooms have been called medicinal mushrooms due to their various morphological, physiological, and ecological characteristics that are also responsible for their diversity [21-25]. Some mushrooms and other fruiting bodies of filamentous fungi are edible and provide a good source of protein, whereas others have narcotic effects and used as medicine. Mushrooms contain many valuable secondary metabolites such as fatty acids, polysaccharides, phenolic compounds, and terpenoids. Mushroom secondary metabolites have different biological activities including, anti-oxidant, anti-viral, anti-inflammatory, anti-coagulate, anti-cholesterol, anti-cancer, antimicrobial activities and other different activities.

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Mushrooms; Enzymes production; Edible mushrooms; Truffles; *Pleurotus spp; Ganoderma lucidum; Agaricus brasiliensis; Flammulina velutipes; Auricularia polytricha; Hericium erinaceus; Agrocybe aegerita; Hypsizygus marmoreus; Lepista luscina; Lyophyllum schimeji; Schizophyllum commune; Coprinus comatus; Inonotus obliquus; Ganoderma applanatum; Grifola fondosa; Lentinus edodes; Lepista luscina; Cordyceps militaris; Cordyceps sinensis; Morchella esculenta*

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Mushroom-associated compounds, applied as therapeutic agents such as anti-aging and skin whitening agents [26-33].

On the other hand, fungi in general and mushrooms in particular are famous producers of different enzymes of industrial applications. Such enzymes have contributed in the efficient role of mushroom in bioremediation and recycling of elements in environment. Hence, we are focusing in this review on the enzymatic machinery reported for some famous mushroom species including the ligninolytic enzymes (lignin peroxidases, manganese peroxidase, and laccases), hydrolases (cellulases, xylanases, pectinases, esterases, amylases, and proteases). Also, we are highlighting their applications in different fields such as dairy, food and feed processing, brewery, leather, textile, paper, agrochemicals, and pharmaceuticals.

Enzymes are one of the key industrial products to be obtained from medicinal mushroom (Truffles and different other edible mushroom genera). In general, the enzymes of fungal origin have advantages over those from other sources due to their higher yield, efficient production using inexpensive media, and the fact that fungal cells act as small factories with a wide variety of catalytic activities [34]. Enzymes have wide applications in a broad range of industries, including food, dairy, confectionary, textiles and leather production. Several truffle species have been investigated for enzyme production, such as *Tuber maculatum*, which produces amylase, xylanase, laccase, lipase, peroxidase, cellulase and catalase and *Tuber aestivum* which produces amylase, peroxidase and catalase) [35].

THE POWERFUL ABILITY OF MUSHROOMS TO PRODUCE ENZYMES

Enzymes from microbial sources are preferred over animal or plant sources due to their ease of preparation, short generation time, ease of manipulation of related genes and large availability in nature. Edible mushrooms are the delicious fruiting bodies of filamentous fungi from Ascomycota and Basidiomycota phylum. Mushrooms are good source of carbohydrates, proteins, fats, vitamins, minerals, enzymes and other bioactive compounds with considerable amount of water and fiber but low in calorie [36]. These edible creatures possess a large group of enzymes including oxidases, lipases, cellulases, lignin degrading enzymes, invertase and proteolytic enzymes depending on the growth condition and substrate utilization [37-39].

There are a lot of edible mushrooms such as *Pleurotus florida, Pleurotus ostreatus, Ganoderma lucidum, Pleurotus cyststidiosus, Agaricus brasiliensis, Flammulina velutipes, Pleurotus eous, Auricularia polytricha, Hericium erinaceus, Agrocybe aegerita, Hypsizygus marmoreus, Lepista luscina, Lyophyllum schimeji, Pleurotus djamor, Schizophyllum commune, Coprinus comatus, Inonotus obliquus, Ganoderma applanatum, Grifola fondosa, Lentinus edodes, Lepista luscina, Piptoporus betulinus, Pleurotus eryngii, Cordyceps militaris, Cordyceps sinensis,* and *Morchella esculenta* which grown on various synthetic and complex media to study their intracellular and extracellular hydrolytic and oxidative enzyme pools [40]. On the other hand, different classes of enzymes reported from edible mushrooms are extensively utilized in various industries such as dairy, food and feed processing, brewery, leather, textile, paper, agrochemicals, and pharmaceuticals etc.

Enzymes are necessary for all the metabolic activities of the mushrooms like hydrolysis, oxidation, reduction and transfer etc. Presence of various enzymes in the mushrooms also contribute to their nutritive value, flavor and shelf-life [41]. Growth of edible mushrooms on substrates and development of fruiting body thereafter are highly dependent on the level of hydrolytic and oxidative enzymes secreted by the mushroom to cleave the organic polymeric constituents of the substrates [38]. Edible mushrooms are reportedly rich sources of various ligninolytic, cellulolytic, proteolytic, lipolytic and stress enzymes

Ligninolytic enzymes

Ligninolytic enzymes are extracellular, oxidative and non-specific enzymes which produce unstable products via oxidative reactions.

Lignin peroxidase: Lignin peroxidases possess hemi containing iron atom in its active site. They catalyze hydrogen peroxide dependent oxidation of non-phenolic aromatic compounds into cation radicals to decompose them. Lignin depolymerization *via* lignin peroxidase is quite efficient because of its high reduction potential [42].

Manganese peroxidase: This is the main oxidizing enzyme of ligninolytic peroxidases. Manganese peroxidase are again heme containing peroxidases that oxidise phenolic compounds to phenoxy radical by oxidation of Mn (II) to Mn (III) ion in the presence of Hydrogen Peroxide (H_2O_2) as a cofactor.

Pleurotus sajor-caju grown on coir pith showed maximum lignin peroxidase and manganese peroxidase activity of about 16.25 U/ml and 20.2 U/ml respectively on 20th day of fermentation with Azolla and Soya hulls as supplement [43]. Initial depolymerization of lignin requires lignin peroxidase and manganese peroxidase, so presence of both the enzymes in reaction mixture will speed up the depolymerization process [42]. On the other hand, Peroxidase's enzymes in general are utilized in various industrial sectors. These enzymes are used to treat textile dyes in textile industry, to degrade herbicides and other wastes in waste treatment plant and to remove lignin from lignocellulosic biomass to prepare animal feed or compost. In food and brewing industry, these enzymes improve quality of animal feed and help in development of flavor, colour and nutritional quality of the food [39].

Laccases: Laccases are blue multi-copper oxidases that catalyze the oxidation of various aromatic substrates along with the reduction of molecular oxygen to water. This enzyme can degrade both phenolic and non-phenolic compounds with oxygen as final electron acceptor [42]. A broad substrate range including polyphenols, methoxy-substituted phenols, aromatic diamines and others with low substrate specificity make them quite suitable for industrial applications [44]. Wild edible mushrooms *Lentinus sajor-caju, Pleurotus giganticus* and *Panus sp.* from Tripura were identified as laccase producers using 0.02% guaiacol as substrate. Also, *Agrocybe aegerita, Agaricus bisporus, Hypsizygus marmoreus, Lentinula edodes, Pleurotus eryngii, Pleurotus ostreatus* and *Pleurotus sajors-caju* were found to be potent producer of laccase in extracellular medium containing copper sulphate along with Tween 80 and 4,6-dimethyl-2-mercaptopyrimidine [44]. Furthermore, *Volvariella volvacea* strain 14, grown on cotton waste produced multiple forms of laccase in submerged culture at 30°C, 85% humidity and alternate 12 hours illumination (499 lux) and darkness before fruiting [45]. Moreover, the edible and medicinally important mushroom *Hericium erinaceus* were cultivated for production of laccase in the growth medium prepared by malt extract and peptone [46]. Laccases are essential for detoxification and decontamination of waste, decolorizing dyes and degrading polyaromatic hydrocarbons in waste management. In paper industry, laccases are used for removing lignin from wood and bio-bleaching of wood pulp. In food industry, laccases help in stabilization of wine, processing of fruit juices and improvement of texture of dough while baking. These enzymes are also required during pre-treatment of lignocellulose, bioremediation of food industry waste water, composting of lignocellulose material, and chemical synthesis of biofuel cells and sensors etc. Laccases are utilized in diagnosis, enzymatic and immunological assays and tests, hair dying and also in reducing effects of poison ivy dermatitis [47].

Hydrolases

Cellulase: Cellulase is a multienzyme complex composed of endo-β-1,4-glucanase, exo-β-1,4-glucanase I and II and β-glucosidase or cellobiase. The enzymes act sequentially to break the linear polymer into monosaccharide glucose unit. The endo enzyme creates random cleavage of internal bonds of the cellulose linear chain. Then exo-β-1,4-glucanase II targets the non-reducing ends of the chain and exo-β-1,4-glucanase II attacks at the reducing ends to produce disaccharide cellobiose [48,49]. The edible mushrooms enzyme production varies greatly depending on the nature of the lignocellulosic materials. In *Pleurotus eryngii* grown on ramie stalks, kenaf stalks, bulrush stalks and cottonseed hull showed ramie stalks and kenaf stalks were best suitable to cultivate the mushroom with biological efficiency of 55% and 57% respectively [50]. Both the substrates induced cellulase enzymes. Also, several commercial strains of *Lentinula edodes* were grown in coffee beans at 25±2°C [51]. Samples were estimated for cellulase activity after 7, 14, 21, 28 and 35 days of incubation. Furthermore, culture filtrate enzymes from *Termitomyces clypeatus* containing β-glucosidase showed comparable efficiency with commercial cellulase during saccharification of carboxymethyl cellulose [48].

Cellulases In textile industry, these enzymes are required for bio stoning of denim garments, improving the efficiency of detergents and cleaning tiles. Cellulase can be used as a potent antitumor agent. In paper and pulp industry, cellulases are used for biochemical pulping, paper recycling and deinking, deinking recycled fibres and improving drainage and runnability of paper mills. Also, these enzymes are vital for preparing easily degradable soft paper and cardboard, improved lignocellulosic biomass for animal feed, residues for improvement of soil fertility and for preparing formulations to remove industrial slime. In food and brewing industry, cellulases are utilized to extract and clarify fruit and vegetable juices, produce fruit nectars and purees, extract olive oil used in carotenoid extractions for production of food colouring agents and prepare additives for the food industry as thickener [47].

Xylanase: The principal component of hemicellulose is xylan which is composed of α-1,4-linked d-xylosyl backbone branched with pentoses, hexoses and uronic acids. Xylanases are debranching enzymes responsible for hydrolysing xylobiose of hemicellulose polymer. Xylanase production was estimated in *Pleurotus eryngii* grown in presence of different concentrations of glucose, xylose, sucrose, starch, xylan, wood straw and rice husk powder as carbon source [52]. Xylanases are essential for complete breakdown of plant biomass comprising of lignin, cellulose and hemicellulose to produce biofuels and other value-added products [53]. In paper and pulp industry, are required for bleaching kraft pulp and deinking printed papers. Also, essential for synthesizing pharmaceutically important xylo-oligosaccharides such as probiotics, anti-inflammatory, anti-hyperlipidemic, anti-oxidant, anti-allergic, anti-cancerous and anti-microbial agents [53]. Furthermore, in food and feed industry, xylanases are used to prepare animal feed for cattle, poultry and fish and also important for clarification of fruit juices and improving rheological property of bread [54].

Pectinases: Pectin is a complex plant polysaccharide composed of esterified D-galactouronic acid residues in an α-(1-4) chain. Pectinases act on pectin to break them down to α-(1-4)-polygalactouronic acid residues. Rate of hydrolysis is dependent on the chain length and higher the chain length, rate of hydrolysis is also high [55]. *Pleurotus sajor-caju* grown on onion waste orduced a thermostable pectinase through solid state fermentation [56]. Also, Pectinase's enzymes were produced by *Lentinula boryana* on wheat straw dextrose agar, 25°C for 7 days [57]. Furthermore, Pleurotus *djamor* var. *roseus* produced amylase enzymes on potato dextrose agar, 25°C for 7 days.

Pectinases are widely used in fruit juice clarification, juice extraction, manufacture of pectin free starch, degumming of natural fibres, treatment of waste water or sewage and in cocoa and tobacco industry. Pectinase treatment increases the rate of fermentation of tea and removes its foaming tendency and the enzyme also removes mucilaginous coat from coffee beans [58]. Proteases hold major share of the industrial enzyme market. Since long these enzymes are used in laundry detergents [59].

Amylases: Amylases are complex group of enzymes that hydrolyses polysaccharides like starch to glucose, thus play a wide range of biotechnological applications in food industry, fermentation, textile and paper industry [54]. Amylases are broadly classified into α, β and γ subtypes. Amylases may act as endo-enzymes which catalyze hydrolysis in a random manner within the starch molecule resulting in linear or branched oligosaccharides of various chain length.

Amylase enzymes were produced by *Lentinula boryana* on wheat straw dextrose agar, 25°C for 7 days [57]. Also, *Pleurotus djamor var. roseus* produced amylase enzymes on potato dextrose agar, 25°C for 7 days.

Esterase: These enzymes are associated with metabolism of a number of therapeutics such as cholesterol lowering drug (lovastatin), anti-infuenza drug (Oseltamivir), narcotic analgesic meperidine (Demerol), cocaine and heroin [60]. This enzyme is also crucial for the enantioselective hydrolysis of esters to produce optically pure compounds or resolution of racemic mixtures by transesterification. Fruiting body of *Hypsizygus ulmarius* produced 2.25 µmole/ml of esterase [60]. Esterase enzymes are involved in ester formation and transesterification with potential application in food, pharmaceuticals and cosmetic industries [60].

Protease: Proteases are indispensable in various industries like dairy, leather, detergent, brewing, meat and photographic industries due its hydrolytic capabilities of amide linkage. Mushrooms are rich in proteases like *Lepista nuda,* and *Termitomyces clypeatus.* Also, proteases from *Lepista nuda* had shown antiproliferative activity on human hepatoma HepG2 cells. Furthermore, protease from *Termitomyces clypeatus* was capable of killing cancer cells, restoring *p*53 level and cleaving surface proteoglycans [37]. Moreover, a novel alkaline and detergent stable protease (SPPS) was isolated from *Pleurotus sajor-caju* strain CTM10057 [59]. On the other hand, edible mushroom *Lentinus citrinus* DPUA 1535 and *Pleurotus ostreatoroseus* DPUA 1720 were investigated for protease production in presence of amazonic tubers Dioscorea trifida, Manihot esculenta and Dioscorea alata supplemented with rice bran or manioc flour residue in varied proportions [61-63]. Each combination was positive for protease but highest titre was achieved from *Pleurotus ostreatoroseus* DPUA 1720 cultivated in *Dioscorea alata.*

CONCLUSION

Mushrooms have prestigious enzymatic machinery allowing their application in different industries; some mushrooms produce highly stable dyes that can be used in textiles and other dye-related products and some mushrooms can be used as Microbial Fuel Cell (MFC). Further studies are needed to elucidate the benefits of mushrooms (Especially Truffles) as enzymes producers.

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