



Brief review: lignocellulolytic enzymes from polypores for efficient utilization of biomass

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Abstract

Polypores are a group of diverse macrofungi that belong to phylum Basidiomycota (Basidiomycetes). The ability of polypores to produce enzymes has attracted the attention of scientists. These enzymes are increasingly being used for many industrial purposes, e.g. textile, pulp and paper, food, detergents and animal feeds. However, their expensive of production is the main hindrance to their use. Fortunately, most polypores can decompose cellulose, hemicellulose, and lignin in the plant cell walls. Solid state fermentation is a good method to degrade lignocellulose and to produce enzymes at low cost since residual agro-industrial waste can be used as the substrate for microorganism growth. Every day large amounts of residues and waste materials are lodged into the environment by industry, agriculture, and other ways leading to environmental problems. The present paper explores the isolation of polypore fungi and their production of lignocellulolytic enzymes, taking full advantage of various agro-industrial wastes as substrate.

Key words – Agro-industrial wastes – Cellulolytic – Fermentation – Fungi – Ligninolytic

Introduction

Enzymes are proteins that are produced by living cells. They are catalysts which control biochemical processes (Robinson 2015). Being proteins, enzymes are biodegradable and can have activity in a wide range of pH values and at ambient temperatures (Gupta 2016). Lignocellulolytic enzymes include two groups; the ligninolytic enzymes which contain peroxidases and oxidases, and the cellulolytic enzymes such as cellulases, hemicellulases, xylanases. Lignocellulolytic enzymes have important industrial and environmental applications. The cost of enzyme production could be significantly lowered by utilizing low-value biological substrates such as agricultural waste (Bharathiraja et al. 2017).

Being a key factor in lignocellulose degradation, lignocellulolytic enzymes such as cellulase and hemicellulase are well known for their applications in various processes. Agriculture biomass wastes contain cellulose, hemicellulose and lignin (Table 1) with smaller amounts of pectin, protein, extractives and ash (Bajpai 2016).

Polypores (basidiomycetes) are a polyphyletic group of wood decaying fungi that are diverse in ecological specificity and morphological characteristics. Most polypores are capable of breaking down lignocellulose, and consequently they play a mainstay role in nutrient recycling in forest ecosystems. Although some polypores can cause tree disease (Ginns 2017), others have medicinal properties (Jayachandran et al. 2017). China possesses the highest polypore diversity in the world with 704 species, belonging to 134 genera, 22 families and 11 orders (Liwei & Yucheng 2013). In

North America 146 genera and 492 species of polypores have been recorded (Zhou et al. 2016). In Seoul, Korea, during 2008–2012, 300 specimens with poroid basidiocarp were collected (Jang et al. 2014). As of 2011, 360 *Polyporales* species from 18 families were recorded in Thailand (Chandrasrikule et al. 2011). Since then, additional polypore fungi have been discovered such as *Polyporus thailandensis*, *Ceriporia cystidiata*, *Macrohyporia dictyopora*, *Perenniporia* sp., and *Ganoderma sichuanense* (Kozue et al. 2016, Ponlada et al. 2016, Ji et al. 2017, Thawthong et al. 2017).

Table 1 Cellulose, hemicellulose, and lignin content in various lignocellulosic biomass

Lignocellulosic biomass	Cellulose (%)	Hemicellulose (%)	Lignin (%)	References
Corn cobs	33.7 – 41.2	31.9 – 36	6.1 – 15.9	Isikgora & Becer (2015)
Wheat straw	49.7	14.8	23.5	Santos et al. (2015)
Corn stover	37.0	22.7	18.6	Kim et al. (2016)
Rapeseed	51.3	17.3	44	Pei et al. (2016)
Bagasse	25.0 – 45.0	28.0 – 32.0	15.0 – 25.0	Putro et al. (2016)
Corn pericarp	22.5	23.7	4.7	Kim et al. (2017)
Grasses	25.0 – 40.0	25.0 – 50.0	10.0 – 30.0	Kumar & Sharma (2017)
Rice husks	25.0 – 35.0	18.0 – 21.0	26.0 – 31.0	Wikee et al. (2017)
Banana peels	9.90	41.38	8.90	Kabenge et al. (2018)
Soya straws	44.2	5.9	19.2	Kim (2018)
Paddy straw	34.8 – 36.4	23.65 – 30.3	10.4 – 11.0	Syazwanee et al. (2018)

Ligninolytic enzymes

Ligninolytic enzymes are elaborated in the utilization and degradation of the complex polymer of lignin as long carbon chain. They involve mainly oxidative enzymes such as laccase (Lac), lignin peroxidase (LiP), and manganese peroxidase (MnP). In recent years, the market for ligninolytic enzymes has risen because of their application in biotechnological areas and their wide use in pollution treatment, for example, degradation of hazardous compounds such as dyes, phenols and xenobiotic (Niladevi et al. 2009). Lignin is a large complex macromolecule which contains three monomers (p-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol) and represents lignocellulosic biomass of approximately 15-25% (Davin & Lewis 2005). In the past 10 years, Thai researchers have become interested in screening and production of lignolytic enzymes from fungi, and research has shown good results. Zecarias et al. (2016) screened ligninolytic enzymes by dye decolorization plate test, with 49 of 61 fungal strains showing decolorizing activity. Vaithanomsat et al. (2010) produced ligninolytic enzymes (Lac and MnP but not LiP) by a white-rot fungus *Datronia* sp. KAPI0039 (Vaithanomsat et al. 2010). Aryl alcohol oxidase (AAO, EC 1.1.3.7) and glyoxal oxidase (GLOX) are accessory enzymes, which take part in lignin degradation. These enzymes are involved in hydrogen peroxide (H₂O₂) production (Tuomela & Hatakka 2011).

Laccases

Laccases (EC 1.10.3.2) are a group of multi-copper enzymes also known as benzenediol, with oxygen oxidoreductase or p-diphenol oxidase belonging to the oxidoreductase class. The reaction of laccase takes place one oxygen molecule was oxidized to water associated with one electron. Oxidation ability of laccase includes a broad range of substrate such as aromatic amines, methoxy-substituted monophenols (Bourbonnais et al. 1995) and polyphenols (Bourbonnais & Paice 1990). This oxidation generates oxygen-centered free radicals that could be converted to quinone in a second enzyme catalyzed reaction (Gianfreda et al. 1999).

Most species of white rot fungi produce laccase to varying degree (Khushal et al. 2010). Laccases play an important role in various industries with applications in cosmetics, food, paper, pulp, textile, synthetic chemistry, environment treatment and pollutant removal agent (e.g., soil bioremediation), biodegradation of phenolic pollutants and removal of endocrine disruptors (Couto & Toca 2006). A variety of agricultural materials have been used for laccase production such as banana peel, bamboo pulp, coffee husks, corn cobs, corn stover, rice husk, rice straw, rice bran, orange peel, and sugarcane bagasse. Research has shown that orange peel is the best substrate for laccase production (Chairin et al. 2013, 2014). Laccase enzymes from polypores have been studied for a long period, especially from common genera such as *Trametes*, *Stereum*, and *Phanerochaete*. Besides these widespread fungi, laccase from *Pycnoporus cinnabarinus* was reported as the only ligninolytic enzyme produced by this species (Khushal et al. 2010). *Trametes versicolor* was found to produce laccase on the third day of cultivation (Osma et al. 2011). *Ganoderma lucidum* has been used as phenolic and metallic inducers to optimize laccase production (Kuhara & Papinutti 2014).

Lignin peroxidase (LiP)

Lignin peroxidase (EC 1.11.1.14), commonly known as ligninase, is one of the most important enzymes involved in the utilization of lignin. Both phenolic and non-phenolic compounds can be oxidized by cleaving the propyl side chain of lignin substructures (Schoemaker et al. 1985) and this enzyme has been shown to depolymerize lignin *in vivo* (Hammel et al. 1993). Oxidation of non-phenolic compounds by lignin peroxidase with a relatively high redox potential have been interpreted by several authors as the result of an unusually high redox potential of the oxidized enzyme intermediates, which are lignin peroxidase compound I (LiPI) or compound II (LiPII) (Hans & Klaus 1996). The enzyme is also able to stabilize the initial product of veratryl alcohol oxidation, the veratryl alcohol radical cation (Valc⁺).

Until recently, LiP enzymes had been reported from only a few white rot fungal genera such as *Bjerkandera*, *Phanerochaete*, *Phlebia*, and *Trametes* (Floudas et al. 2012, Duenas et al. 2013, Riley et al. 2014). Recently, different polypore fungi such as *Ganoderma lucidum*, *Phanerochaete chrysosporium*, and *Bjerkandera adusta* (Kadri et al. 2017, Shaheena et al. 2017, Bouacem et al. 2018), as well as the less studied *Podoscypha elegans* (Agrawal et al. 2017) have been more extracted in lignin peroxidase production ability.

Manganese peroxidase (MnP)

Manganese peroxidase (EC 1.11.1.13) is an enzyme produced by the lignin degraders. MnP shows a strong preference for Mn (II) as its reducing substrate. The redox potential of the MnP – Mn system is lower than that of LiP and normally it does not oxidize non-phenolic lignin models (Glenn & Gold 1985). Generally, lignin polymer provides strength to all higher plants and the natural function of MnP is the degradation of the matrix complex (Sundaramoorthy et al. 1997).

Previous research has suggested that MnP producing polypore fungi such as *Phanerochaete chrysosporium*, *T. versicolor*, *Dichomitus squalens*, *Stereum ostrea*, and *Irpex lacteus*. Sukarta & Sastrawidana (2014) used agricultural waste to increase production of MnP by *Polyporus* sp. The ability to produce MnP by *Phlebia* especially the *P. radiata* group, which produced the highest levels, was shown by Kuuskeri et al. (2015). Mali et al. (2017) asserted that *P. radiata* was the strongest producer of manganese peroxidase when evaluated alone, as well as in co-culture and also *Trichaptum abietinum*.

Cellulolytic enzymes

Cellulolytic enzymes are regularly produced by a wide range of fungi and include cellulases, hemicellulases, pectinases, chitinases, amylases, proteases, phytases and mannases. Fungal cellulolytic enzymes are a group of hydrolytic enzymes responsible for cellulolytic and xylanolytic activities (Mtui 2012).

Cellulases

Cellulases are enzymes that convert cellulose into simple sugars (Chinedu et al. 2005). They are able to hydrolyze β -1, 4 linkages in cellulose chains and are produced by microorganism and animals (Henrissat 1991). The production rate of cellulase from fungi is higher compared to other microorganisms and this can be advantageous (Rana & Kaur 2012). Based on their amino acid sequences and crystal structures, the catalytic modules of cellulases have been classified into numerous families (Henrissat 1991). Generally, complete cellulose hydrolysis is responded by three main types of cellulases combinations: endoglucanases, exoglucanases, and β -glucosidase (Zhang & Lynd 2006). These enzymes are widely used in numerous application areas including beverage, agriculture, paper, textiles, detergent, animal feed as well as an alternative for generating energy. Some organic compounds are inducers for cellulase such as disaccharides, spent ammonium sulphite liquor (Han et al. 2017), and glycerol (Delabona et al. 2016). The production of exoglucanase and β -glucosidase was induced from the co-culture of *Trichoderma viride* and *Ganoderma lucidum* in solid state fermentation (Afzal et al. 2014).

Endoglucanases

Endoglucanase (commonly called CMCase) randomly cut at the β -1, 4-bonds position of cellulose chains and generate new ends for other cellulase combinations to work on. In general, fungal endoglucanases hold a catalytic module with or without a carbohydrate-binding module, while bacterial endoglucanases may retain multiple catalytic modules, carbohydrate-binding modules, and other modules with unknown function (Xiao et al. 2013).

Exoglucanases

Exoglucanases are active at the reducing or non-reducing-ends of cellulose polysaccharide chains, which are then converted to major products either cellobiose or glucose. Exoglucanases work efficiently on microcrystalline cellulose by possible cracking cellulose chains from the microcrystalline structure (Teeri 1997).

β -glucosidase

β -glucosidase or β -D-glucoside glucohydrolase works by degradation of polysaccharides to provide monosaccharide units. These can then be absorbed and used by the organism to hydrolyze short-chain oligosaccharides and soluble cellobiose into glucose. Increasing the length of the cellulose chain causes loss of activity and also performs the hydrolysis of terminal β -D-glucose oligosaccharides as well as biosynthesis of oligosaccharide unit in glycoproteins or glycolipids (Melo et al. 2006). The complex cellulolytic enzymes have a better yield than the individual action when working together (Castro & Pereira 2010).

Xylanases

The synergistic action of xylanase is required for maximizing hydrolysis of lignocellulosic residues (Ghose & Bisaria 1979). The major enzymes are responsible for the endohydrolysis of (1 \rightarrow 4)- β -D-xylosidic linkages in xylans and random endohydrolysis of (1 \rightarrow 3)- β -D-glycosidic linkages in (1 \rightarrow 3)- β -D-xylans. Xylanases are synthesized by many types of microorganisms, marine algae, insects, seeds, etc. but the main source of commercial xylanases is filamentous fungi (Nair & Shashidhar 2008, Dhillon et al. 2011, Chanwicha et al. 2015, Behnam et al. 2016, Amorim et al. 2017). The extracellular xylanase along with several accessory xylanolytic would be released into the medium for disbranching substituted xylans which is one composition in hemicellulose during fungal cultivation. There is significant interest in xylanolytic enzymes for their use as a supplement in animal feed, manufacture of bread, food, textiles, pulp bleaching and in the production of ethanol and xylitol (Polizeli et al. 2005). In Thailand, xylanase has been produced by an endophytic fungus *Alternaria alternata* on biomass substrate (Wipusaree et al. 2011), as well as the overexpression and gene transformation of xylanase by *XylB-2A* gene on *Pichia pastoris* (Roongsawang et al. 2010). A variety of materials have been used for induction of xylanases including pure xylan and xylan-rich

natural substrates, such as sawdust, corn cob, wheat bran, sugar beet pulp, and sugarcane bagasse (Maheshwari et al. 2000).

History of polypores lignocellulolytic enzymes

Table 2 Recent research on lignocellulolytic polypore fungi

Polypore	Enzymes					References
	Laccase	MnP	LiP	Cellulase	Xylanase	
<i>Ganoderma australe</i>	+	+		+		Chuwech & Nuansri 2015
<i>Phlebia radiata</i>	+	+		+		Jaana et al. 2015
<i>Hexagonia hirta</i>	+					Kandasamy et al. 2016
<i>Trametes</i> sp.	+			+	+	Bisht et al. 2017
<i>Cerrena unicolor</i>	+	+				Elisashvili et al. 2017
<i>Ganoderma</i> sp.	+	+				Farradá et al. 2017
<i>Rigidoporous lignosus</i>	+	+				Kantharaj et al. 2017
<i>Irpex lacteus</i>				+	+	Metrevelia et al. 2017
<i>Trametes versicolor</i>	+	+	+		+	Thiribhuvanamala et al. 2017

Bourquelot & Herissey (1897) investigated enzymes from the supernatant of *Polyporus sulphureus* and in 1899, Czapek discovered the lignin-degrading enzyme in *Merulius lacrymans*. In 1906, Buller published a list of enzymes present in the expressed fluid of young fruit bodies of *Polyporus squamosus* growing in nature. In recent years enzymes have been extracted from polypores. Ana et al. (2007) reported laccase production by *Trametes versicolor*, while Vladimir (2008) extracted lignocellulolytic enzymes from *Lentinus edodes*. MnP from *Bjerkandera adusta* was found to be more active than laccase, with much higher MnP activity observed in the culture degrading system (Eichlerová et al. 2007). High laccase activity, but neither MnP nor LiP, was detected in the culture systems of *Coriolus versicolor* cf. *antarcticus* (Levin et al. 2004). Similar results, with high levels of laccase activity but low levels or the absence of MnP and LiP, have been reported by Hatakka (1994), Nyanhongo et al. (2002) for several other ligninolytic white-rot fungi such as *Trametes modesta*, *Junghuhnia separabilima* and *Dichomitus squalens*. Levin et al. (2007) found that after 14 days *Pycnoporus sanguineus* reduced the lignin content of loblolly pine (*Pinus taeda*) chips by 11%. Other species, *Corioloopsis rigida*, *C. versicolor* var. *antarcticus*, *Peniophora* sp., *Phanerochaete sordida*, *Steccherinum* sp., *Trametes elegans* and *Trametes villosa* had lesser effects on lignin reduction. Jo et al. (2011) established that β -glucosidase had more elements than the others for *Ganoderma neo-japonicum* and *G. lucidum*. For comparison, *G. neo-japonicum* had strong β -glucosidase activity and also an ability to produce cellulase (Jo et al. 2011). *Fomes fomentarius* had high activities of both laccase and MnP, and also of celobiohydrolase and 1, 4- β -glucosidase while *Fomitopsis pinicola* produced elevated endoglucanase activity (Tomas et al. 2012). Lignin-degrading peroxidases of *Bjerkandera adusta*, *Phlebia brevispora* and a member of the *Ganoderma lucidum* complex were investigated by Duenas et al. (2013). The lignolytic and lignocellulosic enzymes of *Ganoderma lucidum* were studied by Sasidhara & Thirunalasundari (2014). The results from this research enabled the conclusion that *Ganoderma lucidum* is a good candidate for scale-up production of ligninolytic and lignocellulosic enzymes (Duenas et al. 2013, Sasidhara & Thirunalasundari 2014).

Solid state fermentation

Solid state fermentation (SSF) is a solid matrix that contains less water, hence microbial culture must develop on the surface and within the solid matrix (Barrios 2012, Simeng et al. 2015). Although water is essential for growth of microorganisms, in SSF water can be adsorbed on the solid support or solid matrix. Except for bacteria, SSF is a friendly fermentation and approximates the microorganisms living conditions. Fungi typically grow in nature on solid substrates such as wood,

seeds, as well as dried parts of animals in low moisture condition (Hesseltine 1977). According to Robinson et al. (2001) the benefits of SSF over submerged fermentation (SmF) are high productivity in a brief period, better oxygen circulation, SSF approximates the living environment of the filamentous fungus, there is less downstream processing and wild-type strains of microorganisms can effectuate favorable, energy saving and reduce cost. However, in SSF, process control and scaling-up is more slow-paced than with SmF. At least two types of applications are interested in SSF, lignocellulolytic enzymes production and lignocellulose degradation by itself (Singhania et al. 2010). Solid state fermentation is mainly used for traditional food processing and for fungal enzyme production. Since SSF more closely matches the natural habitat, then enzyme production at the commercial level has been successful with impulse enzyme secretion from fungi (Singhania et al. 2010). In Asian countries, humans have used SSF from ancient times to produce specific products such as “koji” and “sake”. Contrary, in Western countries for the development of penicillin it was shown that SmF is a model technology for fermentation (Pandey 1992). Holker et al. (2004) compared SSF and SmF and concluded that SSF supplies higher volumetric productivities, the substrate is less inhibited, it yields enzymes with a higher temperature, pH stability, and time required is shorter. Roy et al. (2006) reported that for rubber biodegradation, SSF was a better treatment method than SmF. Pérez et al. (2003) showed a detail comparison between the two methods (Table 3).

Lignocellulolytic enzyme application

Wine and brewing industry

In food industry, wine stabilization is the main application of ligninolytic enzymes like laccases (Minussi et al. 2002). In wine production, it is necessary to remove polyphenols because of their unwanted effects and organoleptic features. Many treatments have been used to control this problem, such as enzyme inhibitors, complicated agents, and sulfate compounds to excommunicate discoloration, evaporation and flavour changes. Fortunately, these effects can be prevented and stabilizing beverages can be accomplished through use of laccase (Morozova et al. 2007). In wine production, enzymes improve colour extraction, clarification, and filtration and take full responsibility for quality and stability, for example, amylase, amyloglucosidase, cellulase, glucanases, hemicellulases, pectinases, glucanases, and hemicellulases (Singh et al. 2007, Ghorai et al. 2009). The aroma of wines can be improved through modifying glycosylated precursors by β -glucosidases. Using macerating enzymes to treat grapes for wine fermentation also strengthens the ability of press, settling and juice yields. Cellulases, hemicellulases, galactomannanase, and pectinases are also used in the coffee industry. Enzymes are used from microbial sources such as *Leuconostoc mesenteroides*, *Saccharomyces marscianus*, *Flavobacterium* spp., and *Fusarium* spp. (Binod et al. 2008). Alpha and β -amylase, phospholipase, pullulanase and invertase are used in production of various types of syrups from starch and sucrose and help with product shelf life (Patel et al. 2016).

Pulp and paper industry

(1) Delignification of lignocelluloses

In paper production it is necessary to separate lignin from cellulose fibers, so chlorine, sulphite and oxygen-based oxidants have been widely used. However, these substances are damaging to the environment and they have been replaced by laccase (Kristensen et al. 2008).

(2) Biopulping and biobleaching

Biopulping is the enzymatic pretreatment of wood chips before chemical methods. Chemical bleaching is a fast process but it affects the cellulose in the pulp and the environment is also polluted by various effluents from paper plants. Ligninolytic enzymes are able to delignify and bleach pulp. Although this process is rather slow compared with chemical bleaching, it is an eco-friendly method (Ana et al. 2007). Hakala et al. (2004) chose 86 fungal strains for wood block decay test. Of these, *Physisporinus rivulosus* T241i was the best choice for softwood biopulping application. Behrendt &

Blanchette (1997) used *Phlebiopsis gigantea* to treat wood logs and found that the wood pitch content reduction was promoted, chemical pulping was easier, there was refining energy saving, and better wood ingrain.

Table 3 Advantages and disadvantages of Solid state fermentation over Submerge fermentation (Pérez et al. 2003)

Advantages	Disadvantages
<ul style="list-style-type: none"> • Similar or higher yields • Simple culture media. The substrate usually provides all the nutrients necessary for growth • Low chance of contamination by bacteria and yeast. This allows working in aseptic conditions in some cases • Similar to natural habitats for fungi • Aerification area is high • In case of fungi, inoculation with spores is possible • Simple design reactors with few spatial requirements can be used due to the concentrated nature of the substrates • Low energy consumption in case of vapor treatment, mechanical agitation and aeration are not necessary • Less need for product extraction due to its high concentration. Small volumes of polluting effluents. • Low moisture availability may favour production of specific compounds that may not be produced or may be poorly produced in SmF • In some cases, the products obtained have slightly different properties (e.g. more thermotolerance) • Due to concentrated nature of substrate, smaller reactors can be used to hold the same amounts of substrate 	<ul style="list-style-type: none"> • Only suitable for microorganisms that require low level of water • Substrates require physical pre-treatment • Biomass determination is very difficult • Difficult to monitor process parameters such as pH, moisture content, and substrate, oxygen and biomass concentration • Difficult in agitation (static conditions are preferred) • Spores have longer lag times due to need for germination • High inoculum volumes are needed • Many important basic scientific and engineering aspects are poorly characterized. Information about design and operation of reactors on a large scale is scarce • Contamination by undesirable fungi could happen • May cause difficulty if metabolic heat generated during growth not removed • Extracts containing products obtained by leaching of fermented solids are often of viscous nature • Mass transfer limited to diffusion • Cultivation times are longer

Textile industry

(1) Dye decolorization

The textile industry has a bad reputation as an environmental polluter. Textile waste waters contain various dyes that are hardly decolorized by conventional treatment systems and cause high biological oxygen demand (BOD) and chemical oxygen demand (COD) (Nagaraj & Kumar 2006). Using biological techniques to decolour dye is cost effective and has application for a wide range of dyes. For example, ligninolytic enzymes such as azoreductase, laccases, peroxidases, and polyphenoloxidase can be used to decolorize dyes in waste waters (Imran et al. 2015, Singh et al. 2015).

(2) Denim finishing

In the past, fabric was washed with abrasives to produce a worn or faded appearance in denim before it was partially pre-bleached in sodium hypochlorite then neutralized in a rinsing step. This process was a major polluter of the environment. In 1996, Novozyme (Novo Nordisk, Denmark) took the initiative and “DeniLite” became the first industrial laccase and the first bleaching enzyme which acts with the help of a mediator molecule. Research in this field had led to discovering novel microorganism to produce high quality laccase under normal environmental conditions (Sharma et al. 2005).

(3) Cotton bleaching

Bleaching cotton to reject natural pigments results in flavonoids with white appearance. Hydrogen peroxide was previously used as the bleaching agent, but it damages fibers by decreasing the degree of polymerization. Enzymatic systems not only cause less damage to fibers but also minimize water use and the development of a quality product. Ligninolytic enzymes work at low concentrations without reducing the quality of the fabric (Tzanov et al. 2003). Fungal laccase from *Trametes pubescens* and *Pycnoporus sanguineus* are used in dye decolorization, cotton bleaching and textile application which could be induce the production by copper sulphate and nitrogen source (Spina et al. 2016, Iracheta et al. 2016). Recently, high frequency ultrasound in the range 850 kHz, 400 W has been applied to enhance the activity of laccase and to improve the whitening effectiveness (Gonçalves et al. 2014).

Bio-ethanol production

Fermentation of wood hydrolysates is an essential product for fuel ethanol. Plant cell walls generally constitute 15–40% cellulose, 10–30% hemicellulose and pectin, and 5–20% lignin (Prasad et al. 2007); these are the initial substances for microbes to hydrolyze and can be derived to produce bio-ethanol. The problem of running out of oil, gas and coal and the environmental impacts of fossil fuels is of serious concern (Sun & Cheng 2002). Cellulose hydrolysis occurs on the substrates and endoglucanases are involved. As is well known, white-rot fungi have the capability to degrade lignin (Eriksson et al. 1990). One of the best white-rot fungi is the polypore, *Trametes versicolor*. It secretes enzymes such as phenol oxidase, laccase and peroxidase, which take part in the transformation of aromatic compounds (Lipin et al. 2013). Recently, many fungi have shown efficiency for use as bio-ethanol production by utilizing wastes. These include *Aspergillus niger* (Izmirlioglu et al. 2016), *Ganoderma lucidum* (Bilal et al. 2015), and *Fusarium* sp., *Spicaria* sp., *Rhizoctonia* sp., and *Paecilomyces* sp. (Dar et al. 2016).

Food processing industry

There are numerous applications for using cellulases in food biotechnology. For example, fruit and vegetable juice production requires cellulases to improve extraction methods, clarification, and stabilization, and also xylanases and pectinases have an important application to increase the yield of juices as part of a macerating enzymes complex (Minussi et al. 2002, Carvalho et al. 2008). Enzyme mixtures containing pectinases, cellulases, and hemicellulases are also used for improved extraction of olive oil. Use of macerating enzymes not only improves the cloud stability and texture of nectars and purees, but also rapidly decreases their viscosity (Grassin & Fauquembergue 1996).

Animal feed and agricultural industries

Enzymes were first used commercially in animal feed in Finland in the 1980s. Applications of cellulolytic enzymes can improve nutritional value and absorbance (Dhiman et al. 2002). In feed industry, β -glucanases and xylanases are used to hydrolyze non-starch polysaccharides. By improving digestion and absorption of feed materials, cellulase can significantly improve quality of meat and also weight gain in chickens and piglets (Ramesh et al. 2011). Enzyme use in animal nutrition has become essential following the prohibition of using some food supplements and antibiotics in feed animal product in EU countries (Ali et al. 1995). Many producers supplement feed with enzyme additives to improve the efficiency, decrease the volume of manure produced, reduce cost, lower phosphorus and nitrogen excretion, improve consistency and to help maintain gut health (Michael & Gary 2010). Phytase plays an important role in release of phosphate from phytate, enhancing digestibility. For example, monogastric animals cannot break down the phytate molecules in many plant tissues, such as bran, seeds, cereals and grains to generate phosphorus, which is a key nutritional requirement for animals to provide bone growth. Thus, phytases have been used for animal feed and this has been commercialized in recent years (Blackburn et al. 2015). Lignocellulolytic enzymes consisting of various composition from ligninolytic and cellulolytic have been also tested for enhancing growth of crops and controlling plant pathogens (Ramesh et al. 2011).

Oil extraction

Enzyme treatment is probably the most important in oil extraction as it digests the complex cell wall of oil seeds, altering permeability favoring oil extraction. Enzymes enhance extraction and separation process, eliminate toxic anti-nutritional factors, catalyse carbohydrate, protein and lipid conversion through their antioxidant and biocatalytic activities (Kalia et al. 2001). Enzymes are applied in various oil seed materials such as peanut, sunflower, soybean, grape seed, etc. Using macerating enzymes during olive oil extraction can increase levels of antioxidants and vitamin E extraction (up to 2 kg oil per 100 kg olives) under cold processing conditions. Enzymes also hold back induction of rancidity and oil content in the waste water (Galante et al. 1998). Using enzymes in sunflower oil extraction with non-toxic as solvent showed the highest capacity on antioxidant against peroxy radicals, total phytosterols and omega-3 fatty acids contents, as well as the lowest content of saturated fatty acids, when compared with the oil obtained by conventional methods (Suellen et al. 2016). Interestingly, fungi could be the new representative in developments of biodiesel production using fungal lipases (Aguieiras et al. 2015).

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