ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Role of Fungal enzymes in Polymer degradation: A Mini review.

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ABSTRACT

Extracellular enzymes or exoenzymes are synthesized inside the cell and then secreted outside the cell, where their function is to break down complex macromolecules into smaller units to be takenup by the cell for growth and assimilation. These enzymes degrade complex organic matter such as cellulose and hemicellulose into simple sugars that enzyme-producing organisms use as a source of carbon, energy, and nutrients. Grouped as hydrolases, lyases, oxidoreductases and transferases, these extracellular enzymes control soil enzyme activity through efficient degradation of biopolymers. In their review we are focusing on fungal enzymes and their abilities to degrade polymers to protect environment.

Keywords: Polymers, fungal enzymes, Degradation, Environment.

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ISSN: 0975-8585

INTRODUCTION

There is an increase in hazardous substance emissions to the natural environment that comes with industrial development. The substances emitted into the atmosphere may be a threat to human and animal health as well as cause changes in the troposphere and stratosphere compositions that are difficult to reverse. Transport, petrochemical, coking and chemical industries', including plastics production and processing, dye and varnish production, etc., is the main anthropogenic sources of pollution. Waste dumps are also a significant hydrocarbon source. S substances introduced into the environment are often transformed through various complicated reactions in the air, which may result in an increase in their toxicity [1]. Polymers are recognized as major solid waste environmental pollutants. Another problem is disposal of agricultural plastic wastes. Land filling is the most common method for disposing of municipal solid waste. Many synthetic polymers, resistant to chemical and physical degradation, are produced and utilized. They present disposal problems when their usefulness ceases [2]. Plastics are composed of organic condensation or addition polymers may contain other substances to improve performances. Polymers are derives from Greek words meaning many parts, [3] Plastics are the general term for wide range of synthetic polymerization products. In New Delhi, India generates 5.6 million metric tons of plastic waste annually, with Delhi generating the most of at municipality at 689.5 metric tons every day, according to a report from the Central Pollution Control Board (CPCB).The plastics are extremely durable means that they last a long time for degradation [4]. The plastics have di-2-ethyl hexyls phthalate, which is potential carcinogen. It also contains Cl_2 and will release dangerous toxic material that is xeno-oestrogen. PVC when buried results in emission of deadly named dioxins. Dioxins are considered a carcinogen on the basis of animal studies. The plastics persistence in landfills is adding to the growing water and surface waste litter problems, which has raised concerns about non- degradable products and promoted increased interest in the development of new alternative for the reducing wastes. Table .1 illustrates Industrial uses of different polymer their structures. [5] In developing countries, environmental pollution by synthetic polymers has assumed dangerous proportions. As a result, attempts have been made to solve these problems by including biodegradation of polymers through slight structural modifications. Biodegradation is a process whereby microorganisms such as bacteria, fungi, yeasts and their enzymes consumes a substance as a food source so that its original form disappears[6] . Enzymes are biological catalysts, i.e., they accelerate the reaction rates in living organisms without undergoing themselves any permanent change. In fact, in the absence of enzymes, most of their actions of cellular metabolism would not occur. Biodegradation is a rapid process under appropriate conditions of moisture, temperature and oxygen availability [7]. Biodegradation for limited periods is a reasonable target for the complete assimilation and disappearance of an article leaving no toxins. Many chemical transformation processes used in various industries have inherent drawbacks from a commercial and environmental point of view. Nonspecific reactions may result in poor product yields. High temperatures and/or high pressures needed to drive reactions lead to high energy costs and may require large volumes of cooling water downstream. Harsh and hazardous processes involving high temperatures, pressures, acidity, or alkalinity need high capital investment, and specially designed equipment and control systems [8]. Unwanted by-products may prove difficult or costly to dispose of. High chemicals and

energy consumption as well as harmful by-products have a negative impact on the environment. In a number of cases, some or all of these drawbacks can be virtually eliminated by using enzymes. Enzyme reactions may often be carried out under mild conditions, they are highly specific, and involve high reaction rates. Mild operating conditions enable uncomplicated and widely available equipment to be used, and enzyme reactions are generally easily controlled. Enzymes also reduce the impact of manufacturing on the environment by reducing the consumption of chemicals, water and energy, and the subsequent generation of waste. Enzymes are biological catalysts in the form of proteins that catalyzes chemical reactions in the cells of living or ganisms. As such, they have evolved – along with cells – under the conditions found on planet Earth to satisfy the metabolic requirements of an extensive range of cell types. In general, these metabolic requirements can be defined as:

1) Chemical reactions must take place under the conditions of the habitat of the organism
2) Specific action by each enzyme
3) Very high reaction rates [9]

Fungal Enzymes and their ability to degrade polymers

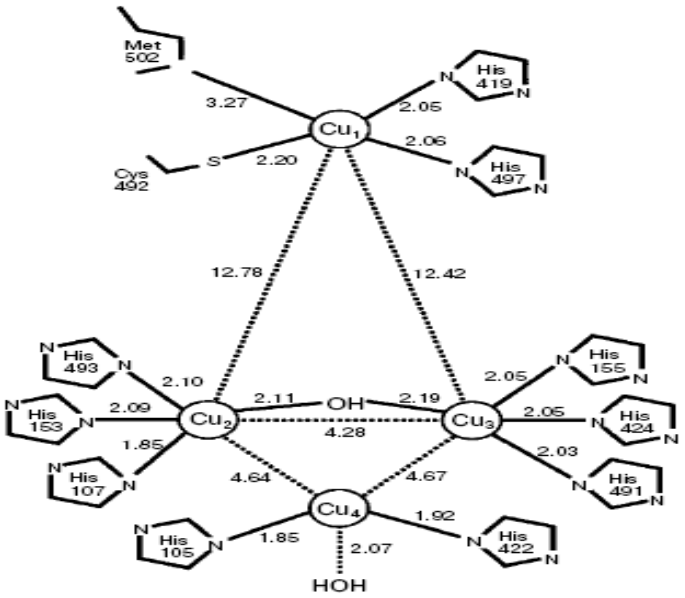
The biological environment in which polymers are present includes the biological agents responsible for the deterioration of polymeric substances. Biological agents such as bacteria, fungi and their enzymes consume a substance as food sources so that its original form disappears. Microorganisms secrete enzymes that break down the plastic polymer into its molecular building blocks which are utilized as a carbon source for growth [10] Fungi are able to degrade a wide variety of polymer [11]. Fungi are nucleated, spore-forming, non-chlorophyllous organisms which reproduce both sexually and asexually; most of them posses‟ filamentous, somatic structures, and cell walls of chitin or cellulose. More than 80,000 species are known. True fungi are present everywhere.True fungi are particularly important in causing the degradation of materials. Their importance as deteriorative agents is a result of materials present in polymer composition. Certain conditions are essential for optimum growth and degradative activity. These include an optimal ambient temperature, the presence of nutrient materials and high humidity.

LACCASES

Laccases (benzenediol: oxygen oxidoreductases, EC 1.10.3.2) are multicopper blue oxidase which are able to oxidize polyphones with oxygen as final electrons acceptor [12]. Moreover, they are also able to oxidize ortho-and aromatic by removing an electron and a proton from a hydroxyl group to form a free radical. Their active site is constituted by four copper atoms and they utilize molecular oxygen as an oxidant for the oxidation of varieties of phenols and other aromatic compounds to corresponding reactive quinines [13]. Laccases are mostly present in lignin- biodegrading fungi, where they catalyze the oxidation of aromatic compounds. Laccase is generally found in higher plants and fungi but recently it was found in some bacteria such as *S.lavendulae*, *S.cyaneus*, and *Marinomonas mediterranea* [14-16]. In fungi, laccase appear more than the higher plants. Basidiomycetes such as *Phanerochaete chrysosporium*, *Theiophora terrestris*, and *Lenzites*, *betulina* [17], and white-rot fungi [18, 19] such as *Phlebia radiate* [20], *Pleurotus*

ostreatus [21], and *Trametes versicolour* [22] also produce laccase. Many *Trichoderma* species such as *T. atroviride*, *T. harzianum* [23], and *T. longibrachiatum* [24] are the sources of laccases. Laccase from the *Monocillium indicum* was the first laccase to be characterized from Ascomycetes which shows peroxidase activity [16]. *Pycnoporus cinnabarinus* produces laccase as ligninolytic enzyme while *Pycnoporus sanguineus* produces laccase as phenol oxidase [25, 26]. In plants, laccase plays a role in lignifications whereas in fungi it has been implicated in delignification, sporulation, pigment production, fruiting body formation, and plant pathogenesis [27, 28]. Laccase activity is known to act on non-aromatic substrates [29] O liveri [30] reported that the olive mill wastewater (OMW) remediation by the extracellular laccase secreted by the white-rot fungus *P. ostreatus.* Laccase produced by the actinomycetes *R.ruber*, involved in biodegradation of polyethylene. Laccase can help in the oxidation of the hydro-carbon backbone of polyethylene. Gel permeation chromatography determine that cell- free laccase incubated with polyethylene helps in the reduction of average molecular weight and average molecular number of polyethylene by 20% and 15 % respectively[31]. Laccases couple the four single electron oxidations of a reducing substrate to the four electron reductive cleavage of the dioxygen bond, using four Cu atoms distributed into three sites, defined according to their spectroscopic properties. Four typical metal content of laccase includes one type-1 copper (T_1), one type-2 (T_2) and two type-3 copper (T_3) ions, with T_2 and T_3 arranged in a trinuclear cluster (TNC). The type 1 site contains the blue copper, whose tight coordination to a cysteine is responsible for an intense SCys → Cu (II) charge transfer transition at around 600 nm, giving the typical blue color to the enzyme. The T_2 shows a characteristic electron paramagnetic resonance (EPR) spectrum, clearly distinct from that of T_1, whereas T_3 coppers are anti- ferro magnetically coupled and EPR- silent ions. T_1 exhibits a planar triangular coordination with the sulfur atom of a cysteine and with the Nδ1 nitrogen of two histidines. The three T_2/T_3 ions are arranged in an triangular fashion and coordinated to a strongly conserved pattern of four His-X-His motifs. 9 Six of such histidine residues coordinate the T_3 copper pair, whereas the T_2 is coordinated by the remaining two histidine residues. Electrons from the reducing substrate are extracted from the T_1, the primary electron acceptor, and then transferred to the TNC through a highly conserved His-Cys-His tripeptide, where the four electron reduction of dioxygen to water takes place. A number of 3D structures of laccases have been solved. All the fungal laccases exhibit a similar molecular architecture organized in three sequentially arranged cupredoxin- like domains.4 The T_1 is located in domain 3, whilst the TNC cluster is embedded between domains 1 and 3 with both domains providing residues for coppers coordination. The distance between the T2 and T3 sites of the enzyme is 4 Å and the T1 copper ion is located at the distance of about 12 Å from them (32,33,34).

ISSN: 0975-8585



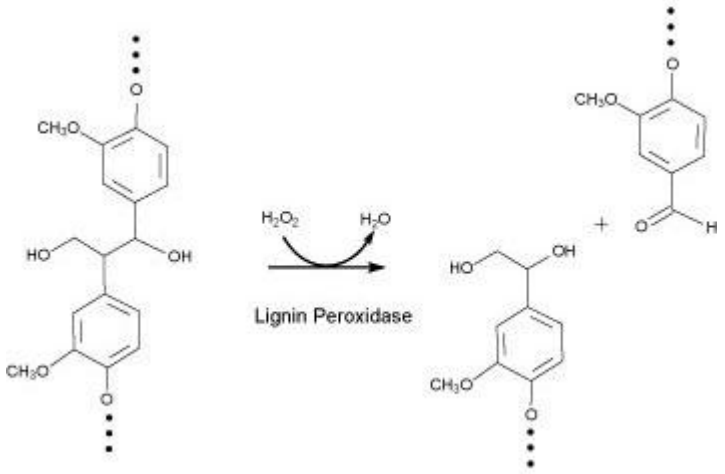
The illustration of the active site of laccase showing the relative orientation of the copper atoms. [33]

Laccases exhibit an extraordinary range of natural substrates (phenols, polyphones, anilines, aryl diamines, methoxy-substituted phenols, hydroxyindols, benzenethiols, inorganic/organic metal compounds and many others) which is the major reason of their attractiveness for dozens of biotechnological applications.10 The repertory of laccase catalyzed oxidative reactions can be enlarged by the means of the so-called mediators. Suitable redox mediators are good laccase substrates, whose oxidized forms have a half- life long enough to permit their diffusion towards otherwise non-oxidable laccase substrates (non-phenolic substrates or large molecules), and possess high oxidation potential to allow laccases to indirectly oxidize them [34]. Laccases may be applied to degrade various substances such as undesirable contaminants, by-products, or discarded materials. Laccase may be applied to degrade plastic waste having olefin units [35, 36]. Likely, an oxidation of the olefin units by the LMS (Laccase Mediator Systems), could initiate a radical chain reaction, leading to the disintegration of the plastic. Also this LMS can be used to degrade polyurethanes [37]. LMS facilitated the degradation of phenolic compounds (environmental hormones) from biphenol and alkyl phenol derivatives [38, 39] and also the decomposition of fluorescent brighteners [40, 41]. A number of applications for laccases have been proposed in several industrial sectors, such as textile, food, paper and pulp, pharmaceutical, chemistry, nano-biotech, cosmetic, along with their application in bioremediation. As a fact, laccases can be adopted [1] to bleach textiles [42] ; (2) To eliminate undesirable phenolics responsible for the browning, haze formation and turbidity development in clear fruit juice, beer and wine [7] (3) To bleach wood pulp [43] ; (4) To synthesize various functional organic compounds such as drug, dyes [44,45] ; (5) To produce various polymers [46] ; (6) to detect molecules in biosensor devices [12] . (7) To produce power in biofuel cells [47]. (8) To dye hair [48]. As far as bioremediation is concerning, laccases may be applied to decolorize textile efflu ents; to degrade plastic waste having olefin units [48] to eliminate odor emitted from places such as garbage disposal sites, livestock farms, or pulp mills; to remove phenolic compounds from olive oil mills and pulp mills wastewaters; [48] and to decontaminate soils from polycyclic aromatic hydrocarbon (PAH) [48].

ISSN: 0975-8585

LIGNIN PEROXIDASE

Lignin peroxidase (LiP, ligninase, diary propane peroxidase; EC 1.11.1.14) is the first oxidative enzyme discovered in *Phanerochaete chrysosporium* [49, 50]. LiP is an extracellular monomeric glycoprotein with a heme group in its active center. LiP has a molecular mass ranges from 38 to 43 kDa and pH from 3.3 to 4.7. It is capable of catalyzing the depolymerization of the aromatic polymer lignin and a variety of non-phenolic lignin model compounds in the presence of H_2O_2 [51, 52, 53 and12].



Catalyzes the oxidative cleavage of carbon-carbon and ether bonds in lignin-related compounds (54)

LiP has the distinction of being able to oxidize methoxylated aromatic rings without a free phenolic group, generating cation radicals that can react further by a variety of pathways, including non stereo specific Cα-Cβ cleavage and β-O-4cleavage in lignin model dimers, aromatic ring opening, oxidation of benzyl alcohols such as veratryl alcohol to corresponding aldehydes or ketones, and hydroxylation of benzylic methylene groups [54, 52, 12]. Ever since the discovery of LiP, veratryl alcohol has played a pivotal role in the study of the lignin biodegradation process. Veratryl alcohol is used as an assay for enzyme activity due to the easily detectable absorbance of the product veratraldehyde at 310 nm. LiP is capable of oxidizing non-phenolic compounds with a relatively high redox potential of the oxidized enzyme intermediates, lignin peroxidase Compound I (LiP I) or Compound II (LiP II) [53]. Polystyrene derivatives by soil micro flora have been reported [54]. Although the recalcitrant nature of lignin impedes its easy conversion, under the right environmental conditions biological systems can transform lignin to various extents [55, 56]. The ultimate transformation of lignin in nature, its complete oxidation to CO_2, is achieved primarily by the white rot basidiomycetes, which are well known for their abilities to degrade lignin [56]. A few groups of organisms are capable of degrading complex lignin polymers, and they are best exemplified by the white rot fungi and others such as *Phanerochaete chrysosporium, Streptomyces viridosporus, Pleurotus eryngii, Trametes trogii, Fusarium proliferatem, Agaricus, Erwenia, Copricus, Mycema, and Sterium.* [57]. Brown rot fungi, in contrast, leave lignin essentially undegraded. There is extensive evidence that incubation with brown rot fungi changes the structure of lignin so that it is increasingly susceptible to biodegradation by other groups of microorganisms [58]. The polystyrene is copolymerization of lignin and styrene monomer increases susceptibility of the resulting lignin-polystyrene product, and particularly its polystyrene

moiety, to fungal degradation. Lignin peroxidase (LiP) activity was measured by the rate of oxidation of veratryl alcohol [59]. Units of activity were equivalent to micromoles of substrate oxidized at pH 3.0 and 25°C to veratrylaldehyde formation [310 nm = 9,300/(M cm)] in 1 min as measured spectrophotometrically[60]. The assay mixture consisted of culture medium filtered through a 0.45-p,m-pore-size filter, veratryl alcohol solution (1 ml, 3 mM, in 0.33 M sod ium tartrate buffer at pH 3.0), and freshly prepared 54 mM H_2O_2(16µl). Lignin-degrading enzymes are one such group of oxidoreductases enzymes, which have practical application in bioremediation of polluted environment [61]. Polyethylene is degraded by *Phanerochaete chrysosporium* and some bacteria like streptomycin Streptomyces viridosporus T7A (ATCC 39115), Streptomyces badius 252 (ATCC 39117), and Streptomyces setonii 75Vi2 (ATCC 39116) and the fungus Phanerochaete chrysosporium (ME 446). [62]

Table 1. Industrial uses of different polymer [5].

Plastics	Uses
Polyethylene	Plastic bags, milk and water bottles, food packaging film, toys, irrigation and drainage pipes, motor oil bottles.
Polystyrene	Disposable cups, packaging materials, Laboratory ware, electronics uses
Polyurethane	Tyres, gaskets, bumpers, In refrigerator insulation, Sponges, furniture cushioning, and life jackets.
Polyvinyl chloride	Automobile seat covers, shower curtains, Raincoats, Bottles, visors, shoe, Garden hoses, and electricity pipes
Polypropylene	Bottle caps, drinking straws, medicine bottles, car seats, car batteries, bumpers, disposable syringes, carpet backings
Polyethylene Tetraphtalate (PET)	Used for carbonated soft drink bottles, processed meat packages peanut butter jars Pillow and sleeping bag filling, textile fibers
Nylon	Polyamides or Nylon are used in small bearings, gears, windshield wipers, and water hose. Nozzles, football helmets, racehorse shoes, inks, clothing parachute fabrics, rainwear, and cellophane.
Polycarbonate	Used for making nozzles on paper making machinery, Street lighting, safety visors, rear lights of cars, baby for house ware. It is also used in sky- lights and the roofs of greenhouses, sunrooms and verandahs. One important use is to make the lens in glasses.
Polytetrafluoroethylene (PTFE)	PTFE is used in various industrial applications such specialized chemical plant, electronics and bearings. It Is met with in the home as a coating on non-stick kitchen utensils, such as saucepans and frying pans.

PROTEINASE

Proteinase (EC 3.4.21.64) is an enzyme that conducts proteolysis, that is, begins protein catabolism by hydrolysis of the peptide bonds that link amino acids together in the polypeptide chain forming the protein. *A. flavus* secretes different classes of proteinases that display elastinolytic activity. The major classes appear to be serine proteases and metallo proteinases [63]. A study in which the *A. flavus* serine proteinase Gene (Sep) was disrupted or if the enzyme itself was inhibited by *Streptomyces subtilisin* inhibitor in *A. flavus* cultures resulted in the increased production of the metalloproteinase (alkaline protease) elastinolytic activity [38]. Apparently, the expression

of genes encoding for serine and metallo proteinases are subject to a common regulatory system that monitors the level of extracellular proteolytic activities. The *A. flavus* metalloproteinase has been purified and characterized [64]. It is secreted as a 35- kD protein, but is very susceptible to catalytic autolysis and readily yields an active, proteolytic 23 kD form of the protein. This 23-kD metalloproteinase is thermo stable and is tolerant of a wide pH range. The purified activity showed severe inhibition by 1, 10-phenanthrolene and ethylene diamine tetra acetic acid (EDTA), confirming it as a metalloenzyme. The isoelectric point was 9.0, confirming it as an alkaline proteinase. Natural protein substrates, in addition to elastin, that are subject to cleavage by this enzyme are cottonseed storage protein, collagen, zein, ovalbumin, and bovine ser um albumin [64], representing a wide range of protein substrates. The principal protein (over 90% of total protein) secreted by *A. flavus* in liquid cultures utilizing either cottonseed storage protein or zein as the sole carbon and nitrogen source is the metalloproteinase [65]. Secretion of this proteinase is subject to catabolite repression by low molecular weight carbohydrates (e.g., glucose, sucrose, etc.). This metallo protease (alkaline) has, apparently; also been isolated from an *A. flavus* isolate derived from a human patient [66], as well as from *A. flavus* var. columnar is, used in the preparation of soy sauce [67]. The high degree of conservation of protease activities among members of *Aspergillus* section *Flavi* suggests an important role for this enzyme in the fungal arsenal of hydrolytic proteins. The 23-kD protease is thermostable, resistant to proteolytic auto degradation, and capable of utilizing a wide range of protein substrates, including both hydrophobic proteins (elastin, cottonseed storage protein, collagen, and zein) and soluble proteins (ovalbumin and bovine serum albumin). These enzymatic properties could prove invaluable in stress- inducing environments (hot and dry) in which these fungi compete with other microorganisms for available resources. Mammal infection plays a minor role in *A. flavus* population increases relative to crop infection and saprophytic growth [68]. However, insect populations represent a significant potentialnutrient resource for *A. flavus* [69,70] where elastinolytic activity would yield selective advantage. Besides nutrient capture, these proteinases might also function in either host invasion or fungal defense. A fair amount of investigative effort has been focused on the proteinases of *A. flavus*, probably due to potential implications in medicine. Variability among *A. flavus* isolates in elastases production suggested strong correlation between isolate ability to express elastinolytic activity and association with invasive aspergillosis in humans [71]. It was also suggested that *A. flavus* isolates causing invasive human lung infections are more likely to produce elastases than are environmental isolates [61]. Elastinolytic proteinases from *A. flavus* have been purified and characterized, and the genes coding for these enzymes have been cloned [66, 68 and 69]. A subsequent survey of *Aspergillus* section *Flavi* that included isolates from *A. flavus, A. oryzae, A. parasiticus, A. sojae, A. nomius, and A. tamarii* revealed strong conservation of elastinolytic potential [64]. In addition,144 isolates of *A. flavus* from six separate regions of the USA all exhibited elastinolytic activity. It was also discovered that borate, a buffer component used in previous studies [72], was toxic to many *A. flavus* isolates [70]. Thus, the subsequent lack of growth of *A. flavus* isolates on this borate-buffered growth medium was interpreted as lack of elastinolytic potential instead of borate toxicity. Additional work has confirmed the strong conservation of proteinase activity in *A. flavus*. Isolates of *A. flavus* obtained from cottonseed, corn, peanuts, insects, and human sources all displayed proteinase activity [73]. Thus, the earlier studies apparently observed an increased incidence of borate

tolerance, not elastinase production, among *A. flavus isolates* causing human lung disease. According to the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology, proteases are classified in subgroup 4 of group 3 (hydrolases) (114a). However, proteases do not comply easily with the general system of enzyme nomenclature due to their huge diversity of action and structure. Currently, proteases are classified on the basis of three major criteria: (i) type of reaction catalyzed, (ii) chemical nature of the catalytic site, and (iii) evolutionary relationship with reference to structure (74). Proteases are grossly subdivided into two major groups, i.e., exopeptidases and endopeptidases, depending on their site of action. Exopeptidases cleave the peptide bond proximal to the amino or carboxy terminial of the substrate, whereas endopeptidases cleave peptide bonds distant from the termini of the substrate. Based on the functional group present at the active site, proteases are further classified into four prominent groups, i.e., serine proteases, aspartic proteases, cysteine proteases, and metallo proteases (75). There are a few miscellaneous proteases which do not precisely fit into the standard classification, e.g., ATP-dependent proteases which require ATP for activity (76). Based on their amino acid sequences proteases are classified into different families (5) and further subdivided into “clans” to accommodate sets of peptidases that have diverged from a common ancestor (77). Each family of peptidases has been assigned a code letter denoting the type of catalysis, i.e., S, C, A, M, or U for serine, cysteine, aspartic, metallo-, or unknown type, respectively. Williams [78] investigated the enzymatic degradation of PLA using proteinase K, bromelain and pronase. The enzyme, proteinase K from *Tritirachium album* was the most effective for PLA degradation. Proteinase K and other serine proteases are capable of degrading L-PLA and DL-PLA but not D-PLA. Furthermore, proteinase K preferentially hydrolyzes the amorphous part of L-PLA and the rate of degradation decreases with increases in the crystalline part [79, 80].

POLYGALACTURONASE

Polygalacturonase (EC 3.2.1.15, pectin depolymerase, pectinase, endopolygalacturonase, pectolase, pectinhydrolase, pectin Polygalacturonase, endo-Polygalacturonase, poly-alpha 1,4-galacturonide glycanohydrolase, endogalacturonase, endo-D-galacturonase, poly(1,4-alpha-D galacturonide) glycanohydrolase, PG) is an enzyme produced in plants which is involved in the ripening process, and by some bacteria and fungi which are involved in the rotting process. PGs degrade polygalacturonan present in the cell walls of plants by hydrolysis of the glycosidic bonds that link galacturonic acid residues. Polygalacturonan is a significant carbohydrate component of the pectin network that comprises plant cell walls [81]. Pectin is a plant-produced polymer of galacturonic acid that is usually highly methylated. Pectin acts a sort of “glue” to hold plant cells into a tissue structure. Endo Polygalacturonase or pectinases are a class of enzymes that hydrolyze long chain pectin’s at internal locations, producing a mixture of molecular sizes. Many plant pathogens secrete pectinases to help macerate host tissues to assist in pathogen entry past host defenses and for nutrient capture. Pectolytic enzymes are wide spread in nature and are produced by bacteria, fungi, protozoa and nematodes [82].

Fungi produce several extracellular enzymes that result in the decomposition of organic matter. One such enzyme is pectinolytic enzymes. The fungus produces these enzymes to break down the middle lamella in plants so that it can extract nutrients from the plant

tissues and insert fungal hyphae [83,8 4]. The members of the fungal genus Aspergillus are commonly used for the production of polysaccharide degrading enzymes. The ability of *A. flavus* isolates to spread between locules of developing cotton bolls (fruits) is strongly correlated with the production of a specific endopolygalacturonase [85]. It was concluded that this specific pectinase (P2C) contributed to aggressiveness in *A. flavus* isolates. It was subsequently discovered that the P2C pectinase is not catabolite-repressed by simple sugars present on cotton lint, a trait that probably aids the function of the enzyme [86]. The two genes that encode polygalacturonases in *A. flavus* (pecA and pecB) have been cloned and characterized [94]. PecB encodes the *A. flavus* pectinases that are catabolite- repressed (P1, P3), while pecA encodes P2C that is not subject to catabolite repression by simple saccharides [87]. Further evidence for the importance of P2C in *A. flavus* invasiveness of cotton bolls is provided by the transformation of *A. flavus* strains lacking P2C with pecA. Such transformants acquire increased ability to cause inter carpellary membrane damage and invade adjacent locules [53]. In addition, removal of pecA 7activity through targeted disruption significantly reduces boll invasiveness [53]. Thus, it appears clear that a specific Polygalacturonase, P2C, is a major factor contributing to maceration of host tissues by *A. flavus* and that regulation of this enzyme is adapted to aid fungal invasion of tissues containing high sugar concentrations. There is a considerable variability in pectinase production by *A. flavus* isolates. *A. flavus* isolates can be characterized as belonging to one of two distinct strains, S and L. Strain S produces both abundant small sclerotia (<400 µm in diameter) and large quantities of aflatoxins, whereas strain L produces fewer, but larger sclerotia (>4 00 µm in diameter) and less aflatoxins. In a survey of 30 isolates of strain S and 57 isolates of strain L that had been isolated in southwest Arizona, it was found that all L isolates produce pectinase P2c, while 50% of the S strain isolates did not produce P2C [88]. It was speculated that S isolates are more closely associated with soil environments where production of pectinase may not be critical for isolate survival, while L isolates are more often associated with plant canopies, where production of pectinase is crucial. Additional polymorphisms were found with respect to pectinase expression in *A. flavus* isolates spread across different geographic regions of the US A. In additional surveys, A. flavus isolates from southwest US A demonstrated the pattern of pectinase expression described above. However, L strain isolates from southeastern USA showed Appl Microbiol, Biotechnol (2007) 77:497–504 499 variables P2c production, while S strain isolates consistently expressed high P2c levels [89].

These observations may reflect differences in ecological niches to which A. flavus is adapted in the two regions. Such geographical polymorphisms with respect to pectinases expression in *A. flavus* isolates have been observed in other global locations. In a survey of *A. flavus* isolates from Zhejiang province of China, no S strain isolates were found [90] and in a similar finding to the expression of S isolates from southeast USA, L strain isolates from Zhejiang province varied in pectinase production, with some failing to produce P2c [90]. From the investigative work completed to date, it appears clear that endo Polygalacturonase are a major factor contributing to tissue maceration by *A. flavus* isolates and also correlate well with isolate virulence in plants. Besides that function, pectinases probably also contribute to nutrient capture, as *A. flavus* can grow on media in which the sole carbon source is pectin [91]. Another hydrolase that probably contributes to tissue maceration is pectin methyl esterase. This activity removes methyl groups from

pectin and has been observed in many *A. flavus* isolates [87, 89]. Pectin methyl esterase presumably aids in the digestion of pectin by opening up more sites for the pectinase enzyme to bind. Highly methylated polygalacturonides are resistant to hydrolysis by pectinases [92]. Despite the prominence of pectinase as a fungal virulence factor, there are probably other factors that play significant roles in successful invasion of plants by *A. flavus*. A survey of S strain isolates from cottonseed (Arizona) taken over several years revealed no correlation between isolate expression of pectinase P2c and aflatoxin contamination of commercial cottonseed [89]. Although P2c-lacking isolates have reduced invasiveness, they still have sufficient pathogenicity to cause aflatoxin contamination of the seed. It is conjectured that failure to see differences in contamination between P2C– and P2C+ S isolates probably reflects the importance of environmental factors in determining the extent contamination. Variation in both times of infection and environmental conditions (i.e., humidity and temperature) to which individual seed are exposed likely because sufficient variation to mask differences observed between the two types of S strain.

CUTINASE

This enzyme (EC 3.1.1.74) belongs to the family of hydrolases, specifically those acting on carboxylic ester bonds. The systematic name of this enzyme class is cutin hydrolase. Cutinase is a serine esterase containing the classical Ser, His, Asp triad of serine hydrolases [82]. The protein belongs to the alpha-beta class, with a central beta-sheet of 5 parallel strands covered by 5 helices on either side of the sheet. The active site cleft is partly covered by 2 thin bridges formed by amino acid side chains, by contrast with the hydrophobic lid possessed by other lipases [93]. The protein also contains 2 disulfide bridges, which are essential for activity, their cleavage resulting in complete loss of enzymatic activity [69]. Cutinases differ from classical lipases, as they do not exhibit interfacial activation. To date, around 40 X-ray structures of Cutinase and its mutants and inhibitor conjugates have been solved [93, 94, 95, 96, 97 and 72]. The stretch Gly-Tyr-Ser-Gln- Gly containing the active site Ser120, has even stronger homology with the consensus sequence Gly-(Tyr or His) - Ser-X-Gly commonly present in lipases. The catalytic triad Ser120, Asp17 5 and His188, is accessible to the solvent and it is located at one extremity of the protein ellipsoid, and is surrounded by the loop 80-87 and by the more hydrophobic loop 180-188 [63]. The above mentioned loops 80-87 and 180-188, bearing hydrophobic amino acids (Leu81, Gly82, Ala85, Leu86, Pro87, Leu 182, Ileu 183 and Val184), may constitute the interfacial binding site [68]. Despite the existence of two side chain bridges of amino acids Leu81 and Val184, and Leu182 and Asn84, the catalytic serine of cutinase is not buried under surface loops, but is accessible to solvent and substrate. The absence of a flap, masking the active-site serine, as in other lipases, probably explains why c utinase is not activated by the presence of interfaces. The binding of cutinase to interfaces seems not to require a main-chain rearrangement, as in the case of lipases, but only the reorientation of few lipophilic side chains, for example Leu81 and Leu182, that play the role of a “mini” flap. Another important feature on cutinase structure is that the oxyanion hole is preformed in Cutinase instead of be ing induced by ligand binding and seems to be stabilized by cutinase Ser42 side chain [94]. Cutinases display hydrolytic activity towards a broad variety of esters, from soluble synthetic esters (e.g. p-nitrophenyl esters) to insoluble long-chain triglycerides. Moreover, cutinases

can be considered as a link between esterases and lipases as they efficiently hydrolyze soluble esters and emulsified triacylglycerols [95]. Therefore cutinase has been evaluated as a lipolytic enzyme in laundry and dishwashing detergent formulations [96, 97 and 98]. Some benefits were achieved with cutinase, when compared with a commercial lipase, LipolaseTM TM, on the removal of triacylglycerols in a single wash process, as cutinase was able to hydrolyze the fats in the absence of calcium [99]. Another interesting application of cutinase is on the degradation of plastics. Polycaprolactone, synthetic polyester, was hydrolyzed to water-soluble products by cutinase from *Fusarium solani f. pisi* [100]. The products of PCL hydrolysis by several fungal PCL depolymerases have been shown to be a mixture of monomers, dimers, and trimers [102]. PCL dimers and trimers are structurally similar to natural inducers of cutinase. We showed that PCL hydrolysis products induce PCL depolymerase synthesis in wild-type *Fusarium* strains but not in the cutinase-negative mutant. Cutinase can be assayed by its activity against esterase substrates [103].and it was found that PNPC esterase activity always paralleled PCL depolymerase activity, except for the low levels of esterase activity observed in mutant cultures and in wild-type cultures grown without cutin or PCL, probably reflecting esterases other than cutinase. Although PNPC esterase activity is a convenient way to monitor cutinase, PNP esters are not specific substrates for cutinase [104]. Specific assay of cutinase has required radio labeled cutin [105].

Conclusion and future prospective

As said earlier, fungal extracellular enzymes are synthesized inside the cell and then secreted outside the cell, where their functionn is to break down complex macromolecules into smaller units to be taken up by the cell for growth and assimilation. Among the different existing fungal enzymes for the degradation of synthetic polymers, laccases have been the subject of study due to their importance in environment protection, where enzymatic catalysis could serve as a more environmentally benign alternative than other enzymes and the currently used chemical processes. In addition, deeper understanding of the biochemistry and molecular biology of laccase will facilitate the development of novel and more economical laccase applications to degrade polymers.

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