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XYLANASES FROM WHITE ROT FUNGI AND ITS INDUSTRIAL APPLICATIONS

[1]HARPREET KAUR, [2]SONU RAHI, [3]A.K. PANDEY AND [4*]DEEPAK K RAHI

[1& 4]*Department of Microbiology, Panjab University, Chandigarh (UT)-160014, India*
[2]*Department of Botany, Govt. Girls College, A.P.S. University, Rewa (MP)-486001, India*
[3]*Department of Biological Science, R.D. University, Jabalpur (MP)-482001, India*
**Corresponding author: deepakrahi10@rediffmail.com*
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Microbial xylanases have attracted considerable recognition, because of their biotechnological prospective in various industrial applications. Xylanolytic enzymes degrade xylans which are main component of hemicelluloses. Of all the hemicelluloses present, xylan is one of the most important and abundantly found polysaccharide having the diverse structures. Due to its structural diversity, xylan is hydrolyzed by a class of xylanase enzymes into its monomeric xylose subunits. These enzymes are produced by a variety of microorganisms but the principle commercial source is wood degrading fungi which is a class of white rot fungi. White rot basidiomycetes degrade lignocellulosic materials by secreting a large amount of extracellular xylanase enzymes. These days xylanase enzyme is used widely in various industrial applications such as a supplement in animal feed, bioremediation, manufacture of bread, production of foods and drinks, pulp and paper industry etc. The present article discusses and illustrates types, properties and potential industrial applications of xylanase enzyme produced by white rot fungi.

Key Words: White Rot Fungi, Xylan, Xylanases, Industrial Applications

Xylan is an important polysaccharide in hemicellulose fraction of lignocellulosic materials. These are the main components of woody plant cell wall and are available in plenty in nature. Potential of Xylan as a renewable biomass substrate for the production of many products such as fuels, solvents and pharmaceuticals have critically been discussed by many researchers (Subramaniyam & Prema, 2002; Rahi & Malik 2016). Enzymatic extraction of Xylan from complex lignocellulosic materials is the first and most essential step in their industrial utilization (Beg *et al*., 2000). Due to the extraordinary potential of fungi especially WRF (wood rotting fungi) to convert complex lignocellulosic materials by their strong enzymatic action have attracted the attention of a large number of workers (Collins *et al*. 2002, 2005; Polizeli *et al*. 2005; Rahi *et al*. 2009, 2018a, 2018b). For most bioconversion processes, xylan must first be converted to xylose or xylo-oligosaccharides. A number of enzymes are secreted by these fungi that are involved in its unique ability to degrade lignin. These fungi have the excellent potential to degrade the lignin (Rahi *et al*., 2009; Wesenberg *et al*. 2003). The very crucial industrial relevance of xylan is its enzymatic hydrolysis (Beg *et al*. 2000). β-xylopyranose are the main subunits of xylan, and for absolute xylan degradation, the chief enzymes involved are endo-1,4-β-D-xylanase (EC3.2.1.8), (Collins *et al*. 2002, 2005; Polizeli *et al*. 2005). Xylanases are produced strongly by fungi especially by white-rot fungi as they they are well known producers of extracellular polysaccharides-degrading enzymes and their level of production of enzymes is higher than other organisms (Rahi *et al*. 2018a, 2018b). The aim of the present article is to discusses the various aspects viz., types, properties and potential industrial applications of xylanase enzyme produced by white rot fungi.

WHITE ROT FUNGI

The white-rot fungus belongs to the division Eumycota (true fungi), subdivision Basidiomycotina, and class Hymenomycetes, subclass Holobasidiomycetidae (Hawksworth *et al*. 1995). This subclass contains nearly all of the wood-decay fungi, as well as the mycorrhizal, litter, and decomposer fungi (Rahi *et al.* 2004, 2005, 2007a, 2007b) It

Table 1: The important species of white rot fungi producing xylanase enzyme.

White Rot Fungi	Family	Order	References
Phanerochaete ostreatus	Phanerochaetace	Polyporales	Khalil, 2002
Phanerocheate chrysosporum	Phanerochaetace	Polyporales	Khalil, 2002
Trametes versicolor	Polyporaceae	Polyporales	Hossain & Anantharaman, 2006
Pleurotus ostreatus	Pleurotaceae	Gilled Mushrooms	Aslam & Asgher, 2011
Pleurotus eryngii	Pleurotaceae	Gilled Mushrooms	Aslam & Asgher, 2011

includes the mushrooms, puffballs, conks, and crustlike fungi. Several well known white rot fungi such as *Phanerochaete chrysosporium, Phlebia radiata Ceriporiopsis subvermispora Pleurotus ostreatus, Coriolus versicolor, Cyathus stercoreus* have been collected and reported from natural forest (Martinez *et al*. 2004; Wan & Li 2012). Amongst these C. *subvermispora* and *P. radiata* have attracted the attention of various workers world over to elicit white-rot decay (Adaskaveg *et al.* 1986). On the basis the whitish appearance of the delignified wood they named as White-rot fungi (WRF) .These are the exclusive familiar organisms efficient enough to mineralise the fractious and heavy heteropolymer lignin (Rodríguez-Couto 2017, Blanchette 1990). During limited nutrient accessibility (nitrogen being the limiting nutrient), the white rot fungi produce various extracellular non-specific enzyme complexes during their secondary metabolism which is the main cause of their activity (Wesenberg *et al*. 2003, Kirk & Farrell 1987). WRF are ubiquitous in hardwood forests and some of them are very specific or selective to degrade lignin present in as hardwood (e.g. birch and aspen) (Bains *et al*., 2006).The lignin present in wood being degraded by white rot fungi make the wood appear white and having fibrous texture. *Phellinus pini*, *Pleurotus* spp., *C. Subvermispora* and *Phlebia* spp., are some species of white rot fungi which degrade wood by favourably attacking lignin more usually than cellulose and hemicellulose. Although *Heterobasidion annosum*, *Irpex lacteus and Trametes versicolor*, are the species of white rot fungi which preferentially decay the cell wall components (Wong 2009). Since they remove lignin leaving the valuable cellulose intact, selective WRF are consider as potential tools for bioindustrial interest (Mitchell & Lonsane 1992). They produces extracellular ligninolytic enzymes viz., laccase, lignin peroxidase and manganese peroxidase that produce H_2O_2 required for peroxidase and xylanases, mannanases, cellulases and phytases activities (Blanchette 1984, 1985). White rot fungi successively depolymerize cell wall substances only to the extent that the products can be utilized consecutively for metabolism (Cowling, 1961). The action of enzymes system of white rot fungi is restricted to the cell wall layers in the immediate vicinity of the hyphae. The important white rot fungi which are able to produce xylanase enzyme are summarized Table 1.

XYLAN

Xylan is the sustainable polysaccharide found profusely on the earth. It forms the major costituent of hemicelluloses which is a composite made up of polymeric carbohydrates including xyloglucan (D-xylose and D-glucose), xylan, arabinogalactan (D-galactose and arabinose), galactoglucomannan (D-galactose, D-glucose and D-mannose) and glucomannan (D-glucose and D-mannose) (Kulkarni *et al*. 1999, Collins *et al*. 2005, Knob *et al.* 2010).). Xylan is believed to be present at the interface between lignin and cellulose, while the other components i.e. xyloglucan,

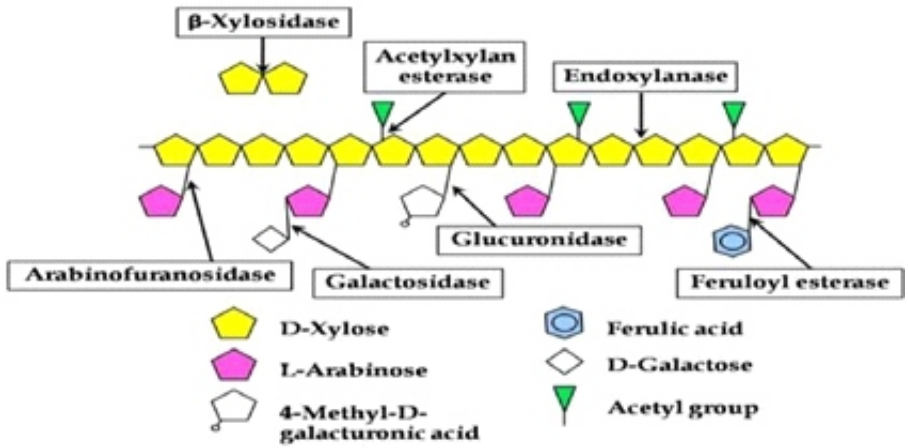

Figure1: Enzymatic Hydrolysis of Xylan *(Corral and Ortega, 2006; Butt et al., 2008; Motta et al., 2013).*

arabinogalactan, galactoglucomannan and glucomannan link using covalent and non-covalent interactions, the four constituents are considered to protect plant cell wall integrity and fiber cohesion (Saha, 2003; Shallom & Shoham 2003). The versatile xylanolytic system is found in many organisms including fungi (Driss *et al*. 2011, Bhardwaj *et al*. 2018).The enzymatic hydrolysis of xylan is done by breaking the glycosidic bonds to form xylooligosaccharides and xylosyl residues by several xylanolytic enzymes such as β- xylosidase, endoxylanase, acetylxylan esterase, α-glucuronidase and α - arabinofuranosidase which come under the super class of hydrolytic enzymes (Fig. 1). Xylans are cleared by the combined action of a group of xylanolytic enzymes. Endoxylanase cleave the xylan into xylooligosaccharides, acetyl eaterases release the acetyl substituents from the β-1,4 D-xylose and β-xylosidase remove xyloses from the non-reducing ends of xylooligosaccharides from the backbone of long chains of xylan.

XYLANASES

Types of Xylanases

The xyloside glycosidic linkage i.e. β-1, are easily hydrolysed by the xylanases enzyme which after replacement of the glycoside by a hydrogen atom forms a sugar hemiacetal and a non sugar compound, the corresponding free aglycone.

Endo-1, 4-β-xylanases

Endo-1, 4-β-xylanases (1,4-β-D-xylan xylohydrolase; EC 3.2.1.8) are the most important xylan degrading enzymes, these enzymes cleave the internal glycosidic linkages of the heteroxylan backbone. The attack of the substrate is not random and depends on the length and the degree of branching of the substrate or the presence of specific substituents within it (Coughlan, 1992). Although endo-enzymes act only at uninterrupted sequences, there are examples of endo-xylanases cleaving the main chain only in the immediate vicinity of a substituted region. Since none of these xylanases liberated the substituent as a free product, this substituent may be required for the proper orientation of the substrate in the active site (Bailey *et al*. 1992). On the other hand, certain endoxylanases, following rigorous purification to apparent homogeneity, not only cleave the main chain but do so only after prior liberation of arabinose. In that respect, endoxylanases have been differentiated according to the end products they release from the hydrolysis of xylan (e.g., xylose, xylobiose and xylotriose, and/or arabinose). Fungal endoxylanases are almost exclusively single subunit proteins with molecular weight values ranging from 8.5 to 85 kDa and isoelectric

point (pI) values between 4.0 and 10.3; most of them are glycosylated (Biely, 1985). Most characterized endoxylanases are optimally active at temperatures ranging between 45 and 75° C, only a small number show maximal activity at temperatures above 80°C (Coughlan *et al*.,1993, Corral and Ortega 2006, Polizeli *etal.* 2005).

β-xylosidases

β-xylosidases (1,4-ß-D-xyloside xylohydrolase; EC 3.2.1.37) can be monomeric, dimeric or tetrameric as per the reports with molecular weight values ranging from 26 to 360 kDa. They are synthesized by various fungi and are believed to be present in the culture fluid, along with the cell, or together (Corral and Ortega, 2006). For both linkage and sugar the xylosidases contain distinct specificity. The unsubstituted β-1,4-linked xylo-oligosaccharides, along with xylobiose and β-xylosides are capable of cleaving by true β-xylosidases. Although, the enzyme shows activity against xylobiose and some against xylan that is almost 30% less than xylobiose (Bailey *et al*. 1992).

α-glucuronidases

This enzyme is responsible for cleaving the glucuronic side chains from appropriate substrates and has been detected in a variety of fungal organisms including white rots (Biely, 1985). α-D-Glucuronidases (EC 3.2.1.131) hydrolyse the α-1,2 linkages between glucuronic acid and xylose residues in glucuronoxylan.

Acetylxylan esterases

Acetylxylan esterases (EC 3.1.1.6) have the capability to remove the O-acetyl substituents at the C2 and C3 positions of xylose residues in acetylated xylans. Few acetylxylan esterases have been purified and characterised until now and little is known about their physicochemical properties. The main reason for the late discovery of these enzymes was the lack of a suitable substrate with which assay them. As described previously, some xylans are acetylated in their native state, although most of the xylans used to study xylanolytic enzymes are deacetylated after alkali extraction (Viikari *et al*. 1986).

INDUSTRIAL PRODUCTION OF XYLANASES

Xylanases are produced by fermentation that can be either submerged (SmF) or solid-state (SSF) (Gouda 2000). However, the maximum production of xylanases are done by submerged fermentation i.e. almost 90% of the xylanases produced and sold worldwide. But the productivity and efficiency of enzyme produced by solid-state fermentation (SSF) is much greater than submerged fermentation. Due to the economic and biotechnological advantages of SSF technique, there is increasing interest in producing highly productive enzyme using this method (Da *et al*. 2005). Also there is an increased demand of production of xylanases from fungal sources due to their high production (Biely, 1985; Sanghi *et al*. 2008). SSF process poses several advantages over SmF which includes lesser chances of contamination, increased enzyme productivity and stability, enzyme produced has more specific activity, production of protein rich byproduct, low fermentation cost, mimicks the natural mode of fungus and much easier steps of downstream process of the enzyme extraction and recovery (Polizeli *et al*., 2005). Since fungi are able to grow at relatively low water activities, SSF conditions are more preferable in contrast to most bacteria and yeast, for which submerged fermentation conditions are more suitable. On the other side, the culture conditions such as pH, temperature, incubation time etc are better controlled in submerged fermentation in comparison to solid state fermentation (Kumar *et al*. 2017). The aerobic microorganisms undergo submerged fermentation which is a familiar and widely available method for the production of xylanase due to more nutrients availability, sufficient oxygen supply and less time required for the fermentation (Senior *et al*. 1989;

Dashtban *et al*. 2010).

APPLICATIONS OF XYLANASE

Bioremediation of environmental pollutants

The pollution of soil, water and air caused by toxic chemicals is degrading environment quickly and is a major need of concern these days. These chemicals are very hazardous and can cause many life threatening diseases harming human health and environment. Hence the toxic compounds need to be eliminated from the environment. However, the available methods and techniques for removing these carcinogenic compounds is rather expensive and stagnant and mostly unproductive and can produce dangerous pollutants (Grassi *et al*., 2011; Bushra *et al*., 2015). In this sense, the use of xylanases produced from white rot fungi seems a good perspective. Since chemical structure of lignin and many toxic pollutants share similar chemical structures such as alkanes, fuels, pesticides, polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), synthetic dyes and explosives, the need to the use of xylanases from white rot fungi for the decay of these toxic contaminants has been examined (Paszczynski *et al*. 1991). Since a combination of many pollutants cause environmental problems, the use of these fungi is highly recommendable (Mester & Tien 2000). White rot fungi can make the use of lignocellulose because of their survival in broad range conditions of environment. Moreover, white rot fungi promote the growth of the indigenous micro-organisms which further help in the breakdown and decay of these pollutants.

Pulp and paper industry

Cellulose from lignin must be separate for pulp and paper production. It can be done by either mechanical or chemical methods. In chemical method, chemicals are used to solubilise lignin which then forms a brown residual substance which is necessary to remove to obtain white paper. These days' delignification using oxygen and hydrogen peroxide is done, but conventionally elemental chlorine was used. However, the whiteness and brightness of paper is achieved less using current method of delignification. Prior to tradition pulping methods i.e. chemical or mechanical, the wood fragments are treated with enzymes produced by white rot fungi is well known biopulping. Xylanases from white rot fungi reduces the use of chemicals and energy consumption so they have been considered as prospective useful means for biopulping, thus the process is environment friendly. Also, biopulping reduces the pollutant toxicity and also removes a few wood extractives (Ali & Sreekrishnan, 2001). Besides, ligninases and hemicellulases (xylanases) have their use in biobleaching. Viikari *et al*., (1986) studied the use of xylanase in the reduction in chlorine consumption. The enzymatic treatment makes the pulp more accessible to oxidation by the bleaching chemicals. A minor effect is due to the enzymatic hydrolysis of the residual non-dissolved hemicellulose by endoxylanases. The residual lignin in unbleached pulp (Kraft pulp) is linked to hemicellulose and the cleavage of this linkage will allow the lignin to be released.

Textile Industry

Many plant fibres such as linen and hessian can be processed by xylanases enzyme.but xyalanase must be free contain cellulose fibres. Cellulose fibres can be liberated by incubating xylanases enzyme with dried ramee stems (Bruhlmann *et al*. 2000; Csiszar *et al*. 2001; Prade 1995). The textile industry needs to be explored more for the use of xylanse enzyme.

Valorization of lingo-cellulosic wastes

The lignocellulosic waste cumulated from various human activities is a matter of concern these days which leads to major environment problems (Dias *et al*. 2010). The lignocellulosic material is composed of cellulose mainly along with lesser amount of hemicellulose and lignin. The cellulose and hemicellulose are made up of various sugars which are macromolecules while lignin built

from phenylpropanoid is an aromatic compound. These components differ in plants in proportion and composition (Sanchez *et al*. 2009). The decay of lignin is not an environmentally friendly process either by physical or chemical method. These waste materials can be converted into value added products with the use of xylanases from white rot fungi.

Ruminant feed

The lignocellulosic wastes are hard to digest by animals due to the presence of lignin linked with cellulose and hemicelluloses (Arora & Sharma 2009). Various physical and chemical methods are in use nowadays to degrade lignin as it is not easy to use by gut microbes of rumen (Chaturvedi & Verma 2013). The use of enzymes from white rot fungi is considered as a good substitute in place of physical or chemical methods as they can lead to the production of various environmental contaminants. The arabinoxylans present in the animal feed are degraded by xylanases enzyme which further make the raw material less viscous (Twomey *et al*. 2003).Preferably these days specific lignin degraders are used to transform lignocellulosic waste into ruminant feed which degrade only lignin and left behind the entire cellulose intact. However this has been experimented only on small scale using single substrate or fungal species. This needs further validation and optimization to outgrow the traditional method (Kuijk *et al*. 2015). Addition of xylanase in animal feed helps in better animal growth rates by improving digestibility and quality of animal litter (Ali & Sreekrishnan 2001, Arora & Sharma 2009). The cereal grains have good quantity of polysaccharides in their endosperm cell wall in the form of arabinoxylans mixed with linked glucans, celluloses, mannans, and galactans out of which arabinoxylans and glucans constitute major portion.

Production of wine

Xylanases from white rot fungi is used to break down xylans, pectin and hemicelluloses present in fruits into simpler molecules such as xylose and glucose (Elisashvili *et al.* 2009). By breaking down cell wall, it helps extract more juice from the fruit which further helps in wine production.

Improvement of bread quality

Enzymes play a key role in baking industry and xylanase has been reported to have its use in bread making. The rheological properties of dough, crumb firmness and specific volume of bread improved considerably using enzymatic hydrolysis of non starch polysaccharides (Courtin & Delcour,2002). When xylanases enzyme is employed in optimum quantity it improves dough stability, dough machinability, loaf volume, oven spring and crumb structure (Butt *et al*. 2008). Bread quality improves by increasing specific bread volume. When both amylase and xylanase are combined the quality further enhances (Das *et al*. 1984). Xylanases increases the elasticity of the gluten network, hence used as additives within the bake loaf volume. Increased elasticity improves handling and stability of the dough.

6.8 Bio ethanol production

The increase in crisis of non-renewable fossil fuels, researchers are searching for a good alternative. The lignocellulosic biomass which is widely available and rich in carbs can be used as a raw material for ethanol production which can replace the conventional fuels. The lignocellulosic waste must be pretreated before ethanol production and this pretreatment is done by white rot fungi very efficiently. The white rot fungi which have been studied for this purpose are *Trametes versicolor, Cyathus stercoreus, Pleurotus ostreatus* and *Phanerochaete chrysosporium* (Wan & Li, 2012, Knezevic *et al*. 2013). The bio ethanol produced from this is sustainable and eco friendly than the conventional fuels used.

Degumming

Xylanase along with pectinase enzyme is used for degumming of many phloem fibers such as jute, hemp, flax, cotton, sisal and bast (Garg *et al*. 2012, Voragen *et al*. 1992). For this, xylanse

must be free of cellulolytic enzymes. Both pectinase and xylanases are involved in removing the binding materials from the plants. This combination is also used in wood processing (Wong & Saddler 1997).

Fruit juice clarification

Fruit juices obtained after extraction are turbid, viscous and cloudy. This turbidity in juices is due to various polysaccharides in juices such as pectin, lignin, starch, cellulose and hemicelluloses. The interactions among haze active proteins and polyphenols result in more turbidity on cold storage of juices known as haze formation (Siebert 1999). Xylanases in combination with pectinase and cellulase enzymes is used in fruit juice clarification. These enzymes improve the quality, yield and clarity of fruit juices that increases the shelf life of the juice (Biely 1985).

Xylooligosaccharide and Xylitol Production

The most recent application of xylanases is for the production of xylooligosaccharides. They are produced from xylan and have 2-6 xylose subunits linked via β –(1-4)-xylosidic linkages. These oligosaccharides are widely used in many industrial and biomedical applications. Their major application is in probiotics and prebiotics. They are also used in pharmaceuticals, agricultural purposes, and feed formulations and as food additives (Vazquez *et al.* 2000). They have low clarific value and are non carcinogenic. Xylitol produced from xylose which is product of hydrolysis of xylan is an anti-diabetic agent which can be used as sweetner for diabetic patients (Nigam and Singh, 1995).

CONCLUSION & PROSPECTS

Xylanases present enormous possibility in various industrial and biotechnological areas and research fields such as bioremediation, pulp and paper, animal feed, food industry, hemi-cellulosic wastes etc. Considering the world market in enzymes, more novel enzymes with high yield and enhanced application ranges will make the use of enzymes in future more ruthless. Since the future seems very bright with the use of newer technologies but there is also a need for search of newer fungal species belonging to white rot group within the great biodiversity found in our country especially in its tropical forests. The wide adaptability of fungal strains to survive at extreme environmental conditions can be exploited for their potential applications. Such strains can be isolated from biotopes with varied set of environmental conditions. Other alternative is to isolate the gene responsible for xylanase production from the suitable and potential strain/s using molecular biotechnology and then transported to expression system. For enhanced production and desirable industrial application, the expression system of these organisms can be regulated. The availability of recombinant DNA technology and genetic engineering along with the use of bioinformatics, proteomics, metabolomics and genomics can be used for higher xylanase yield. However, the only hinderance in the whole process is imitation of natural environment into synthetic. This needs to be taken care of for large scale production and potential use. The ethical issues need to be cleared first so that there is no harm caused due to these technologies to the mankind and other living beings.

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