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## REVIEW ARTICLE

## Review on fungal xylanases and their applications

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## Abstract

Xylanase is responsible for hydrolysis of xylan, a major hemicellulose of plant cell wall. Xylanases are produced by different kinds of microorganisms like bacteria, fungi, protozoans and some yeast. But the level of xylanase in fungal culture is typically much higher than those from yeasts or bacteria. There is an increasing demand for cost effective microbial xylanolytic enzyme which benefits the industrial applications and are produced commercially. Solid state fermentation offers advantages over submerged fermentation especially for fungal cultivars. The major uses of xylanase are biopulping, bleaching, clarifying fruit juices, production of biofuels, in baking industries and textiles.

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## INTRODUCTION

There has been growing interest in xylanase production and its application because, xylanase is important in the bioconversion of hemicellulose, which is a significant component of lignocellulosic material. Xylanase is a class of enzymes produced by microorganisms to breakdown a component of plant cell walls known as hemicellulose.

## Xylan

Xylan is a heteropolymer and it is a second most abundant biopolymer after the cellulose and the major hemicellulosic polysaccharide found in the plant cell wall (Timell, 1967). It is the most abundant non-cellulosic polysaccharide present in both hardwoods and annual plants and accounts for 20–35% of the total dry weight in tropical plant biomass. In temperate softwoods, xylans are less abundant and may comprise about 8% of the total dry weight. Xylan is found mainly in the secondary cell wall and is considered to be forming an interphase between lignin and other polysaccharides. It is likely that xylan molecules covalently link with lignin phenolic residues and also interact with polysaccharides, such as pectin and glucan. In simplest forms, xylans are linear homopolymers that contain D-xylose monomers linked through  $\beta$ -1, 4-glycosyl bonds. In nature, they are partially substituted by acetyl 4-o-methyl-d-glucurono-syl and l-arabinofuranosyl residues, forming complex heterogenous and polydispersed polymers. Many structural aspects of xylans are unclear because of the difficulties associated with the isolation of xylans from natural raw materials without significant alteration or loss of the original structure and association with other components (Joseleau *et al*, 1992).

Xylan displays a large polydiversity and polymolecularity. This corresponds to their being present in a variety of plant species and to their distribution in several types of tissues and cells. All land plant xylans are characterized by  $\beta$ -1,4-linked-D-xylopyranosyl main chain, which carry a variable number of neutral or uronic monosaccharide substituent or short oligosaccharide side chains.

## Xylanase

Xylanase is the name given to a class of enzymes which degrade the linear polysaccharide  $\beta$ -1,4-xylan into xylose, thus breaking down hemicellulose, which is a major component of the cell wall of plants (Lee *et al*, 2003). Xylanases play important physiological role in plant tissue, because they are involved in fruit softening, seed germination and plant defense mechanisms (Turner *et al*, 2007).

Due to the heterogeneity and complex chemical nature of plant xylan, its complete breakdown requires the action of a complex of several hydrolytic enzymes with diverse specificities and modes of action. Thus, it is not surprising for xylan-degrading cells to produce an arsenal of polymer-degrading proteins (Kapporet *et al*, 2001). The xylanolytic enzyme system that carries out the xylan hydrolysis is normally composed of a repertoire of hydrolytic enzymes, including endoxylanase (endo-1,4- $\beta$ -xylanase, E.C.3.2.1.8),  $\beta$ -xylosidase (xylan-1,4- $\beta$ -xylosidase, E.C.3.2.1.37),  $\alpha$ -glucuronidase ( $\alpha$ -glucosiduronase, E.C.3.2.1.139),  $\alpha$ -arabinofuranosidase ( $\alpha$ -L-arabinofuranosidase, E.C.3.2.1.55) and acetylxyylan esterase (E.C.3.1.1.72). All of these enzymes act cooperatively to convert xylan into its constituent sugars. Among all xylanases, endoxylanases are the most important due to their direct involvement in cleaving the glycosidic bonds and in liberating short xylooligosaccharides (Vermaet *et al*, 2012).

Based on their amino acid sequence similarities, xylanases are mainly classified into families 10 and 11 of the glycoside hydrolases. GH10 xylanases generally have a molecular weight  $\geq 30$  kDa and a low pI, while GH11 xylanases are generally smaller (approximately 20 kDa) and have a high pI (Watanabe *et al.*, 2014).

## Fungal xylanases

The fungal genera *Trichoderma*, *Aspergillus*, *Fusarium*, and *Pichia* are considered great producers of xylanases (Adsulet *et al*, 2005). White-rot fungi have also been shown to produce extracellular xylanases that act on a wide range of hemicellulosic materials. For example, *Phanerochaete chrysosporium* produces high levels of  $\alpha$ -glucuronidase (Castanares *et al*, 1995) and *Coriolus versicolor* produces a complex xylanolytic combination of enzymes (Abd El-Nasser *et al*, 1997).

Although xylanases from eubacteria and archae bacteria have considerable higher temperature optima and stability than those of fungi, but the amount of enzyme produced by these bacteria is comparatively lower than that produced by fungi. In general, the level of xylanase in fungal culture is typically much higher than those from yeasts or bacteria (Singh *et al*, 2003).

In the fungal kingdom, a majority of both xylanase and  $\beta$ -D-xylosidase enzymes producing organisms belong to the genus *Aspergillus*, many of which have been well characterized. Filamentous fungi which are main producers of this enzyme from an industrial point of view due to extracellular release of xylanases, higher yield compared to bacteria and yeast and production of several auxiliary enzymes that are necessary for debranching of the substituted xylanase. The attention on the applications of xylan degrading enzymes has led to discovery of many new enzymes with novel characteristics from various microorganisms (Wang *et al*, 2003).

Thermophilic fungi, a unique group of microorganisms, that thrive at high temperatures are often associated with piles of agricultural and forestry products and other composting materials (Maheshwari *et al*, 2000). The distribution and colonization of thermophilic fungal population in compost is closely related to their ability to produce a variety of cell wall degrading enzymes. Since these fungal strains function in amelioration of xylan substrate present in lignocellulosic waste, each xylanase produced may be biotechnologically important and show specialized function. Alkaline-active xylanases of thermophilic fungi find application in bleaching of pulp in paper industry obviating the need for chlorine (to some extent) in ecofriendly process (Subramaniam *et al*, 2002).

## Xylanase Production

Xylanases are produced by either solid state or submerged fermentation. Enzyme production in solid state fermentation (SSF) is usually much higher than that of submerged fermentation. Therefore, solid state fermentation has gained interest from researchers in recent years and has often been employed for the production of xylanases because of economic and engineering advantages (Sonai *et al*, 2005). Solid state fermentation can be performed on a variety of lingo-cellulosic materials, such as rice bran, wheat bran, ragi bran, corn cob, soya bran etc., which proved to be highly efficient technique in the production of xylanase (Pandey *et al* 1999). Solid state fermentation (SSF) is an attractive method for xylanase production, especially for fungal cultivations, because it presents many advantages such as- the higher productivity per reactor volume as well as the lower operation and capital cost.

The cost of carbon source plays another major role in the economics of xylanase production. Hence, an approach to reduce the cost of xylanase production is the use of lignocellulosic materials as substrates rather than opting for the expensive pure xylans (Senthilkumare *et al*, 2005).

## Industrial applications

Xylanase has aroused great interest recently due to its biotechnological potential in many industrial processes, for example, in xylitol and ethanol production, in the cellulose and paper industry, in the production of oligosaccharides, to obtain cellular proteins, liquid fuels and other chemical substances, in the food industry and in poultry, pork and caprine feeding (Eriksson, 1985).

Enzyme conversion of most of the agricultural wastes requires that both cellulose and hemicelluloses be converted to their component sugars. Agro-industrial and food-processing wastes are available in staggering quantities all over the world, which largely become a source of health hazard. The utilization of these wastes for the production of strategic chemicals and fuel requires hydrolysis of all the components. Because xylan is a major plant structural polymer, xylanases and the microorganisms that elaborate them could be used in food processing and paper and the pulp, sugar, ethanol, feed and agro fiber industries (Wong *et al*, 1988).

The process of ethanol production from lignocellulosic biomass includes delignification of the plant biomass and hydrolysis of cellulose and hemicelluloses to monosaccharides. The hydrolysis process can be performed by treatment with acids at high temperatures or by enzyme action. The acid hydrolysis requires significant energy consumption and acid resisting equipment, which makes the process more expensive. The enzymatic hydrolysis does not have these disadvantages. Because of the complex composition of lignocellulosic biomass, the synergistic action of several enzymes (endoglucanases, EC 3.2.1.4;  $\beta$ -glucosidases, EC3.2.1.21; xylanases, EC 3.2.1.8 and  $\beta$ -xylosidases, EC 3.2.1.37) is needed for complete hydrolysis (Howard *et al*, 2003).

Another major area of application of xylanase is the use for the bleaching of kraft pulp in the pulp and paper industries. Most xylanases known to date are optimally active at or below 50°C and at acidic or neutral pH 6 (Viikari *et al*, 1994). In the process of enzyme-assisted pulp bleaching, the incoming pulp has a higher temperature and an alkaline pH, making the use of thermostable alkaline xylanases very attractive (Gessesse *et al*, 1998). Xylanase has been used in bleaching during the paper production, resulting in reduced use of chemicals and resulting in a better brightness (Lemos *et al*, 2000).

In combination with pectinases and other enzymes, xylanases have also been used in other processes such as clarification of juices, extraction of coffee and extraction of plant oils and starch (Eriksson, 1985).

Xylanases have potential applications in the food, animal feed, paper, pulp and biofuel industries (Khandeparkar *et al*, 1986). In feed formulations, cooperation of xylanases, glucanases, proteinases and amylases reduces viscosity of the feed and increases the adsorption of nutrients. Enzymes liberate nutrients either by hydrolysis of non-degradable fibers or by liberating nutrients blocked by these fibers (Lemos *et al*, 2000).

The application of xylanolytic enzymes has increased for the last few decades owing to their potential effectiveness in breadmaking (Butt *et al*, 2008). Starch and non-starch carbohydrate hydrolyzing enzymes are commonly used in the bread making industry as bread improvers (Javier *et al*, 2007). Xylanases make the dough more tolerant to different flour quality parameters and variations in processing methods. They also make the dough soft, reduce the sheeting work requirements and significantly increase the volume of the leavened pan bread (Harbak *et al*, 2002).

The xylanolytic complex can also be used in the textile industry to process plant fibres, such as hessian or linen (Csiszár *et al*, 2001).

Fungal xylanases have great potential and industrial benefits. Xylanase with other enzymes can provide benefits for the industries.

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