Chapter

Xylanases: A Biotechnological Potential Enzyme

D Gowdhaman* and V Ponnusami

School of Chemical & Biotechnology, SASTRA Deemed to be University, India

***Corresponding Author:** D Gowdhaman, School of chemical & Biotechnology, SASTRA Deemed to be University, Thanjavur, Tamil Nadu, India, Tel: +91 9842581181

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Abstract

The second most widely distributed natural polysaccharides present in the plant cell wall of both hard wood and soft wood plants are xylan, which is one of the natural resources on the earth. Depending upon the side chain groups in various position of the homopolymer back bone, the xylan structure varies among the different plant species. Xylanases are a set of co-ordinatively acting enzymes required for complete degradation of xylan. These xylonolytic potential enzymes hydrolyse polymeric xylan to its derivatives like monomeric and dimeric saccharides like xylo-oligosaccharides by the action of permease enzyme into the cytoplasm and the initiate the expression of endoxylanase and β-xylosidases, where β-xylosidase liberate pentose sugar xylose from xylo-oligosaccharides. Due to variety industrially important applications, a major research attempt has been made towards the screening of novel microorganisms which exhibit the interesting characteristics and properties in order to meet the need of an industry. Native enzymes are not sufficient to meet the demand, due to low yields and incompatibility of the standard industrial fermentation processes. Therefore, molecular approaches must be implemented to design xylanases with the required characteristics. Genetic engineering and recombinant DNA technology allow the large-scale expression of xylanases in homologous or heterologous protein-expression hosts. As industrial applications require cheaper enzymes, the elevation of expression levels and efficient secretion of xylanases are vital for ensuring the viability of the process. As a result, there are continuously endeavors to transform xylanase industry into a profitable market with lower costs of production using different types of agricultural extracts at the optimal growth conditions.

Introduction

According to Pharmaion report titled "India Industrial Enzymes Market Forecast and Opportunities, 2020", by 2020 the market for industrial enzymes in India is estimated to exceed 361 million USD.

This growth in the market owes to rise in industrial processing and manufacturing services. Global market for enzymes was calculated as $4.2 billion in 2014 and estimated to expand at a compound annual growth rate (CAGR) of around 7 % over the period from 2015 to 2020 to reach nearly $6.2billion [1].

Enzymes are extensively use in various industries viz., food, feed, pharmaceutical, textile, paper and pulp bleaching etc. With increase in demand for constant innovation towards developing and adopting clean and green technologies, use of microbial enzymes assumes paramount significance. Enzymatic processes helps in production of desired and specific products and thus the issues pertaining to undesired by-products can be easily circumvented. Studies are also carried out in developing enzymes that can work under operating conditions such as different temperatures and acidic or basic pH conditions [2]. Apart from being eco-friendly, enzymatic processes results in better product quality at reduced manufacturing cost with less waste generation and would require less per capita energy consumption [3]. In contrast, conventional synthetic processes would result in large amounts of undesirable by product posing challenges in their separation or disposal. Microbial enzymatic activity can be tuned to suit any given process by careful manipulation of external variables such as enzyme dose, temperature etc. Enzymes being biocatalysts and specific in their action, the amount of enzyme used are relatively very less.

Among various microbial enzymes xylanase is one which has wide variety of industrial applications including the degradation of polymeric xylan to biofuels and industrially important chemicals [4]. Xylooligosaccharides obtain through the degradation of polymeric xylan by xylanases are used in food and feed applications [5]. There is a growing interest to increase pH and thermal stability of the enzyme. Further, there is also an incentive to develop processes that can better hydrolyze soluble and insoluble xylans. There are various wild-type xylanases with preferred properties such as pH stability and enhanced activity. However, there are no single xylanase that has catered to all the needs of an food processing industries. Furthermore, industries need low cost enzymes. Hence, increase of expression level and the competent production of xylanases are vital to bulk production at lower cost. Given this scenario, strain improvement using genetic engineering tools play a significant role in mass production of xylanases with preferred properties.

Xylan

Xylan is the second abundant structural polysaccharides next only to cellulose and it is present in the lignocellulosic materials, especially in plants [6] xylan is mainly constituent in lignocellulosic materials secondary cell wall. It is bound to cellulose (1,4-ß-glucan) and lignin through covalent and non-covalent interactions. Xylans presence in plant cell wall is significant association of fiber and its complexity. Its structure is comprised of homopolymeric support made up of β-1,4 glycosyl bonds [7].

Xylan can be used to produce numerous value added products including natural food sweetner xylitol. It is also used in treatment of life style disorders like diabetics [8]. Arabinoxylans, Glucuronoxylans, Glucuronoarabinoxylan and Galacto-glucuronoarabinoxylans are the class or families of xylan which is abundant naturally [8].

Enzymes in Xylan Hydrolysis

Several enzymes and their mode of actions are necessary for the total conversion of xylan, because of the heterogeneity and complicated in character. Figure 1 explains the complex structure of the xylan is completely hydrolyzed to hexose and pentose monomers by different enzymes together with endoxylanase (endo-1,4-β-xylanase, E.C.3.2.1.8), β-xylosidase (xylan-1,4-β-xylosidase, E.C.3.2.1.37), α-glucuronidase (α-glucosiduronase, E.C.3.2.1.139), α-arabinofuranosidase (α-Larabinofuranosidase, E.C.3.2.1.55) and acetylxylan esterase (E.C.3.1.1.72) [8]. Above listed enzymes work together to hydrolyse xylan into its sugar derivatives.

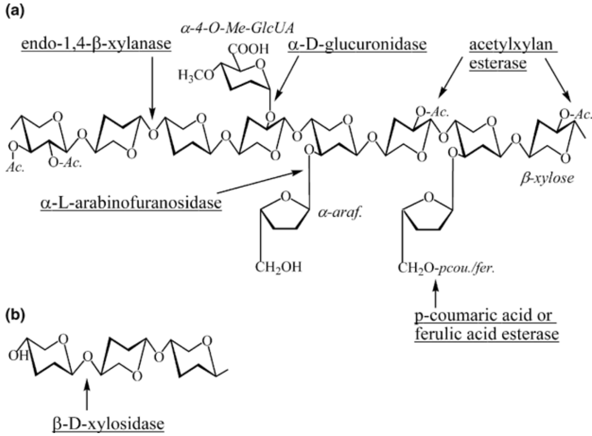

Figure 1: Enzymatic hydrolysis of xylan by various xylanolytic enzymes. (a) endo-1,4-β-xylanase (b) β-D-xylosidase. Adapted from Sunna, A., Antranikian, G. [9].

Xylanases

Among various xylanases, endoxylanases (E.C.3.2.1.8) is one of the enzymes used in the biodegradation of xylan. Biohydrolysis of xylan is in a complex process in which the β-1,4 glycosyl bond is hydrolysed for the liberation of xylooligosaccharides (XOs) (14). Xylan is high molecular weight polymer. Hence, it cannot penetrate into the plant cell with ease. On the other hand, low molecular weight XOs molecules such as xylose, xylobiose and xylopentose can easily enter the cell wall and plays a important function in biosynthesis of xylanase [10].

Carbohydrate-Active Enzyme (CAZy) database [11] contains rationalized content on the description, properties and categorization of xylanase enzymes. There are many families of glycoside hydrolase (GH) xylanases. Primarily, xylanases are classified as GH10 and GH11. This classification is based on catalytic domains and their cluster analysis and based on the sequence similarity of amino acids [12].

GH 10 and GH 11 Families

GH family 10 is mainly consist of endo-1,4-ß-xylanases and endo-1,3-ß-xylanases (EC 3.2.1.32). These families enzymes have low molecular weight and pI of around 8–9.5 [13]. The enzymes belonging to this family can cleave the aryl ß-glycoside linkage of xylobiose and xylotriose. Xylanase is belongs to the Family 10 and posses 4 or 5 sites for substrate binding [14]. These enzymes have high molecular mass and a low pI value [4]. In comparison to other xylanases, GH11 enzymes display enhanced activity and enzyme stability; they are relatively smaller in size and can function under diverse temperature and pH conditions. These characteristics make them ideally suitable for variety of industrial applications and hence, these are also termed as "true xylanase" [13].

Applications

Though xylanases constitute most of the commercially sold hemicellulases, it accounts only a small fraction of the total enzyme sale. With increase in attention to the potential use of these enzyme in different industrial processes, it is expected that sale of these enzymes will increase in future [8]. A few of the important applications of xylanase enzymes are mentioned in Table 1. The less common and recognized application includes its use in brewing and detergent, preparation of coffee, antimicrobial agent production and antioxidant, rayon, cellulose ether and cellulose esters [14-17].

Table 1: Industrial applications of microbial xylanases [7].

Market	**Industry**	**Function**	**Reference**
Food	Fruit and vegetable processing, brewing, wine production.	Xylanases enhances the juice extraction quality. It reduces the viscosity and improves the fruit juice yield. Overall process performance and product quality was also found to improve with the use of xylanase.	20,21
	Baking	Flexibility and potency of the dough are improved with the use of enzyme. This resulted in easier handling and enhanced process capacity. Improvement in bread texture was also reported	21, 22
Feed	Animal feeds	Xylanases decreased the content of non-starch polysaccharides and resulted in decrease of intestinal viscosity. Digestibility was also found to increase. The Nutrition level of less hydrolysable lignocellulosic feed materials like barley and wheat was also found to increase with the use of enzymes.	20,23
Technical	Paper and pulp	Chlorine consumption and toxic discharges was decreased with the use of enzymes.	24
		Xylanase was found to facilitate the pulping process. This resulted in need for mechanical pulping methods and thus energy consumption was also reduced.	20
		Method competence and the paper potency was improved owing to improvement in characteristics property of pulp	20
		Use of xylanase facilitated the de-inking method and reduced the amount of alkali required for the de-inking process.	20
	Starch	Use of enzyme was found to decrease batter viscosity and enhance gluten agglomeration	22
	Textiles	Chemical retting methods can be reduced / replaced by use of xylanase enzyme	24
	Bioremediation/ Bioconversion	Waste water treatment and reusuability of waste materials. Production and manufacturing of industrially important biofuels and chemicals.	25

Need for Strain Improvement

To meet the precise needs of an industry, xylanase enzyme should possess certain ideal characteristics and properties like thermal stability, pH stability, halo stability, solvent stability, higher enzyme activity and enhanced yield [18], in addition to cost economic production [19]. But most of the wild type xylanase demand from natural sources does not possess all of these characteristic properties necessary for industrial applications [17]. Figure 2 represents the characteristics of ideal enzymes. The desired characteristics of an ideal enzyme, as shown in Figure 2, includes the characteristics features includes enzyme activity and thermal stability at broad range of pH and temperatures, substrate specificity, selectivity and rate of conversion of substrate to maximize the yield of product.

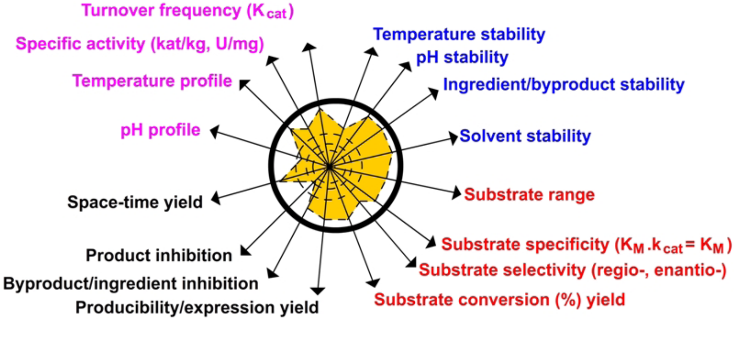

Figure 2: Searching for the ideal enzyme and need for strain improvement. Adapted and modified (Lorenz & Eck 2005).

To overcome the disadvantages with the native enzymes, researchers have adopted various strategies and molecular approaches to enhance the characteristics of xylanases through the methods like heterologous gene expression, protein engineering, for the production of recombinant xylanase, which exhibit better characteristics than the wild type enzymes.

Genome Shuffling- An Evolutionary Engineering

The conventional strain improvement methods for the selection and screening of mutant strains with improved activity and characteristics have been practiced for several years. A substitute to conventional strain enhancement method is genome shuffling. The general scheme of genome shuffling was presented in Figure 3, in which the process of genome shuffling consist of two steps, includes the creation of parental library by isolation and fusion of protoplast and the required phenotype has been selected [26].

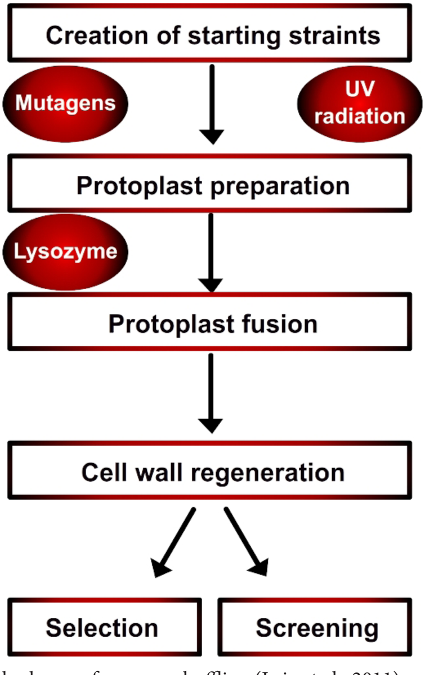

Figure 3: A general scheme of genome shuffling (Leja et al., 2011).

The significance of this method is the genetic propagation can be carried out on the tested microbes with no information of its genetic background, creating it an extremely successful process without the need for its genetic information. This method is also widely used to enhance various important industrial strains such as *Lactobacillus, Sphingobium chlorophenolicum, Streptomyces and Nocardia* sp [27-31].

Moreover, the use of 2-deoxyglucose (2DG), a glucose analogue, as an anti metabolite has been routinely practiced for selection of the hyper producing mutants and recombinants after every round of genome shuffling [32].

Xylanase involved in various industrially important applications. However the major drawback lies in commercial utilization of xylanase due to high cost. Development of cost effective process is prerequisite for the utilization of xylanase in various applications.

Conclusion

Xylanases have broad range of application in various industries such as paper and pulp, food and feed and Biopharmaceutical. To meet specific industrial needs, an ideal xylanase should have specific properties, such as stability over a wide range of pH values and temperatures, high specific activity, and strong resistance to metal cations and chemicals. Other specifications include cost-effectiveness, eco-friendliness, and ease of use. Therefore, most of the reported xylanases do not possess all of the characteristics required by industry. As industrial applications require cheaper enzymes, the elevation of expression levels and efficient secretion of xylanases are vital for ensuring the viability of the process. As a result, there are continuously endeavors to transform xylanase industry into a profitable market with lower costs of production using different types of agricultural extracts at the optimal growth conditions.

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