# Xylan and xylanases: characterization, and biotechnological application in biomass conversion

Tahani Alqahtani<sup>1</sup>, Reda Amasha<sup>1</sup> and Magda M. Aly<sup>1,2\*</sup>

<sup>1</sup>Department of Biology, College of Science, King Abdullaziz University, Jeddah, Saudi Arabia. <sup>2</sup>Botany and Microbiology Department, Faculty of Science, Kafrelsheikh University, Egypt.

# Abstract

Xylans are polymers of xylose which is a residue of pentose sugar with side branches formed through aglucuronic acids. Xylan form cross-linking with cellulose and lignin thus, xylanolitic product can be classified into three groups. Many studies reported degradation of xylan by the production of xylanase using different microorganisms like bacteria, fungi, and yeast. Bacillus species are one of the most prolific producers of xylanolytic enzymes, like Rhodothermus marinus, Streptomyces sp., Stenotrophomonas maltophila, Termotoga thermarum and Bacillus halodurans. Xylanase immobilization enhances enzyme activity as well as eliminates the problems associated with free enzyme systems in industrial applications. Different Xylanases have different properties depending on their source characters and molecular weight, making them very useful for different industrial and biotechnological applications. In conclusion, agricultural wastes are increased every year and can be degraded by microbial enzymes which can be used in different fields and remove agriculture wastes.

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# I. Introduction

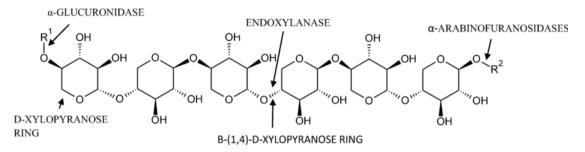
On the earth, the most abundant organic sources are plant-based products which are essential for many industrial purposes like the production of many products. Regarding the cost and yield of enzymes in biotechnology, cellulases and xylanases production are very important and need more studies. Thus, renewable sources can use for the production of higher enzyme titers which will be an addition in the field of biofuels and degradation of agriculture byproducts like corn cobs which are composed of a significant level of lignocellulose and hemicellulose content (Kumar *et al.*, 2008, Knob *et al.*, 2014). The consumption the abundant agricultural wastes increase the nutrition value without causing environmental pollution (Kumar *et al.*, 2017).

Xylose is a monomer produced from xylanases action on the hemicellulolytic part of lignocelluloses. These enzymes were identified as a group of extracellular hydrolyzing enzymes which degrade xylan, the second most abundant carbohydrate after cellulose. Xylan is a polymer composed of D-xylopyranosyl units linked with thermo and acid-labile  $\beta$ -1, 4 glycoside linkage (Khusro *et al.*, 2016). For the hydrolysis of hemicelluloses, the use of strict conditions causes the production of unwanted products. Therefore, saccharification enzymes that degrade carbohydrates polymer are essential for hemicelluloses degradation without toxic materials. On the large scale, xylanases production for potential industrial uses is increasing and gaining much importance to decrease the enzyme cost by using of high yielding microbes (Ramanjaneyulu and Rajasekhar Reddy, 2016). Using pure xylanase is recommended in many fields like making wine, bread, and papers in addition to animal feed, fruit juice, and fine textile manufacture (Chadha *et al.*, 2019). Many studies reported the production of xylanase using different microorganisms like bacteria, fungi, and yeast (Ergün and Çalık, 2016, Kumar *et al.*, 2018, Chadha *et al.*, 2019) either singly or in combination, co-culture of more than one organism (Yardimci and Cekmecelioglu, 2018). Bacteria and fungi have been established as effective xylanase producers and so many of them have been reported to produce xylanase.

# Xylan presence and structure

Plants produce the most renewable biological carbon polymers on the earth within their secondary cell walls. Cellulose, hemicelluloses, and lignin compose the walls of these plants. Despite their significance as main load-bearing constructions for plant growth and their industrial importance as materials and energy sources, it is still unclear exactly how these elements are arranged inside the cell wall. Cell wall macro fibrils that are the common feature of the native hardwood and softwood samples had cylindrical structures with diameters exceeding 10 nm. A similar construction of the secondary cell wall detects in *Arabidopsis thaliana*, which allows comparing the macro fibrils with mutants which dissimilar in cellulose, hemicellulose, and lignin structure. Results indicated that the macro fibrils in *Arabidopsis* cell walls depend on the simple biosynthesis or structure of lignin, cellulose, or xylan (Lyczakowski *et al.*, 2019). Figure 1 showed the fine structure of xylan

which are present in the cell wall of some plants, With the improvement of sustainable energy, corn cob offers significant potential as a renewable organic resource. Xylanases enzymes are essential for the efficient conversion of biomass into valuable products.



## Figure 1. Xylan structure and xylanolytic enzymes involved in its degradation (Suhag and Singh, 2019).

#### Xylanase characters

Xylan hydrolysis by extracellular enzyme xylanase produces imperative products like xylooligosaccharides (xylose, xylobiose) (Ahmed *et al.*, 2016). There have been reports of it in a wide variety of bacteria and fungi as well as *Bacillus, Staphylococcus, Cellulomonas, Micrococcus, Streptomyces, Aspergillus, Penicillium, Fusarium, Pseudomonas, Rhizopus, Clostridium, Trichoderma.* For xylan degradation, various enzymes of xylanases need to act synergistically. The enzymes that responsible for the degradation of the main polymer chain are endo-1,4- $\beta$ -xylanase (EC 3.2.1.8),  $\beta$ -xylosidase (EC 3.2.1.37) and  $\alpha$ -arabinofuranosidase (EC 3.2.1.55), acetylxylan esterase (EC 3.1.1.72), and  $\alpha$ -glucuronidase (EC 3.2.1.139), which work on the degrading side substituents from heteroxylans (Rogalski *et al.*, 2001, Burlacu *et al.*, 2016a).

## Sources of xylanases

Many prokaryotes such as bacteria, cyanobacteria, fungi, yeast, and marine algae produce xylanase (Annamalai *et al.*, 2009, Mandal, 2015). Xylanase also has been isolated from various other sources, including the anaerobic bacterium Clostridium acetobutylicum, immature cucumber seeds, and germinating barley seeds. In spite of this, fungi and bacteria are the most common sources of this enzyme. (Sizova *et al.*, 2011, Burlacu *et al.*, 2016b). The evolution of enzyme species may result in strains with different thermostability properties as a result of evolution. Cellulase-free xylanases effective at high temperatures and pH are of increasing significance in the pulp and paper industry (Kumar *et al.*, 2018).

#### Xylanase from bacteria

*Bacillus* species are one of the most prolific producers of xylanolytic enzymes, like *Bacillus* sp., *Bacillus halodurans* (Gupta *et al.*, 2015), *Bacillus pumilus* (*Thomas et al.*, 2014), *Bacillus subtilis* (Banka *et al.*, 2014), *Bacillus amyloliquefaciens*, *Bacillus circulans*, and *Bacillus stearothermophilus* (Chakdar *et al.*, 2016). Several bacteria in the extreme environment produce xylanase which has cold and high-temperature adaptability and pH stability. Bacillus species has been reported to produce thermotolerant xylanase active at very high temperatures of 60–70 °C (Thomas *et al.*, 2014), *Bacillus Halodurans* TSEV1 (Kumar and Satyanarayana, 2014), *Rhodothermus marinus* (Karlsson *et al.*, 2004), *Streptomyces* sp. (Sukhumsirichart *et al.*, 2014), *Stenotrophomonas maltophila* (Raj *et al.*, 2013), *Termotoga thermarum* (Shi *et al.*, 2013). Because of many benefits, namely low cost, ability to work at different pH and temperatures, and catalytic activity, bacterial xylan hydrolysis is the best. Furthermore, xylanase with salt and ethanol tolerance is a good candidate for generating xylooligosaccharides from alkaline-extracted xylan (Torkashvand *et al.*, 2020).

The solid-state fermentation method can examine and optimize xylanase production using agricultural wastes such as sugarcane bagasse. *Bacillus substilis* subsp. Subtilis JJBS250 produced the highest amount of xylanase (20.35U/g) (Singh, 2020).

#### Xylanase from fungi

The major source of hemicellulases such as glucanases and xylanases comes from fungi. Because of their ability to produce thermophilic enzymes, thermophilic fungi are of great commercial importance. These thermophilic fungi can flourish at a temperature of  $40-60^{\circ}$ C. In addition, they have higher enzyme kinetics and better thermostability, as well as less chance of contamination and better storage capacity (Latif *et al.*, 2006).

Some species of thermophilic fungi can produce low molecular weight and thermo-alkali-stable xylanase. Extracellular xylanases produced by thermophilic fungi are thermostable and have a wide pH tolerance. They are highly resistant to denaturing causes and are optimal at higher temperatures (Kalogeris *et al.*, 2003, Li *et al.*, 2006, Moretti *et al.*, 2012). In contrast, these fungi's xylanase activity is typically inhibited by metal ions, like mercury ions (Bajaj and Abbass, 2011, Boonrung *et al.*, 2016). Hence, it is necessary to explore new sources of xylanases with properties that can apply in the industry (Seemakram *et al.*, 2020).

Functionally diverse xylanases Various species are produced from *Trichoderma*, *Aspergillus*, and *Penicillium* (Pal and Khanum, 2010). Based on the carbon source used, Isoforms of the enzyme and dissimilar xylanases in the culture medium and their patterns were identified by (Gonzalez-Vogel *et al.*, 2011). endo-1,4-  $\beta$ -xylanases (EC 3.2.1.8),  $\beta$ -D-xylosidases (EC 3.2.1.37),  $\alpha$ -L-arabinofuranosidases (EC 3.2.1.55),  $\alpha$  glucuronidases (EC 3.2.1.139), acetyl xylan esterases (EC 3.1.1.72), and ferulic/coumaric acid esterases (EC 3.1.1.73) (Gomez del Pulgar and Saadeddin, 2014) are important for the lignocellulose degradation and they can be purified and characterized for their potential application in industries.

## Xylanase from Actinomycetes

In lignocellulose degradation, particularly xylan, actinomycetes are considered the important group of microorganisms for degradation (Burlacu *et al.*, 2016b). Related to xylanase production, Actinomycetes have been considered to a lesser extent, Although they are generally regarded as a great source of primary and secondary metabolites (McCarthy, 1987(Kansoh and Nagieb, 2004, Rawashdeh *et al.*, 2005).

Fungi and actinomycetes have been examined as the origin of enzymes that hydrolyze organic material, particularly agricultural wastes. Soils are a major source for isolation of new species of Actinomycetes which are isolated from numerous different sources. As a result of their highly varied secondary metabolism, producing a significant number of antimicrobials, antitumor agents, and many enzymes, they are essential in biotechnology (Da Vinha *et al.*, 2011). Many Streptomyces species produce enzymes that contribute to cellulose, hemicellulose, and lignin breakdown (Nascimento *et al.*, 2009).

Streptomycetes can break down many macromolecules such as proteins, cellulose, starch, lipids, and chitin. Many Streptomyces sp. strains can produce xylanase and cellulase that degrade cellulose, hemicellulose, and lignin, but the enzymes are mainly thermolabile (Jang and Chen, 2003).

Thermo-alkaline stable xylanase has been produced by Streptomyces sp. They can thrive efficiently at low cost and with cheap substrates, such as wheat bran, can bagasse, corn cobs. In addition to producing more effective strains, submerged fermentation of Streptomyces sp. allows optimization of processes and increases automation level. Recently, Xylanase derived from bacteria is more interesting because of the biotechnological application of enzymes. Such applications need xylanases with specific properties (Coman *et al.*, 2011). Under solid-state fermentation, *Streptomyces geysiriensis* used to production extracellular xylanase from agricultural wastes like rice bran and sawdust (Poornima *et al.*, 2020).

# Immobilization of xylanase

Immobilization of xylanase enhances enzyme properties and activity as well as eliminates the problems associated with free enzyme systems in industrial applications. Many studies have been used entrapment and covalent binding with calcium alginate beads to immobilize xylanase. Immobilization conditions such as enzyme loading, agitation rate, and glutaraldehyde concentration affect xylanase yields (Sukri *et al.*, 2020).

## Application of xylanase

Xylanases have significant applications in all fields of the industrial enzyme market. In commercial applications, xylanases produced by microorganisms are preferred because of their biotechnological characteristics (Basit *et al.*, 2020). Since there is a large amount of lignocellulosic biomass that is abundant, renewable, and ever-present, it can use to manufacture paper and pulp, chemicals, fuels, and other products by using microorganisms (Bala and Singh, 2016, Singh *et al.*, 2016).

#### Agro waste treatment

Xylanase enzyme can convert Hemicellulose (Xylan) in agro-waste into Xylose. With the development of an advanced enzymatic hydrolysis process, new possibilities for hemicellulosic waste disposal are possible (Biely *et al.*, 1985).

The most common organic substance in nature is lignocellulose biomass which consists of an interconnected structure of cellulose and hemicellulose combined with glycosylated proteins and lignin polymer (Wang *et al.*, 2011). Feedstock can degrade by microorganisms isolated from nature and the guts of herbivorous animals. Large animals cannot degrade lignocellulosic materials themselves, and instead, they depend on their guts microbial communities (Dar *et al.*, 2015).

#### Biofuels

It was found that the production of simple sugars from a polysaccharide like cellulosic and Lignocellulosic wastes or any economical agricultural waste materials like wheat bran, sugarcane bagasse, crushed citrus fruit peel, hay, and corn cob had an increasing interest due to the produced biofuel, bioethanol and/or bio-based materials (Zhao and Yang, 2011). The conversions of hydrolytic microbial enzymes are mainly endoglucanase, cellobiohydrolases, xylanases, and  $\beta$ -glucosidase (Tao *et al.*, 2010, Maitan-Alfenas *et al.*, 2015). Hardwood hemicellulose is composed of 15 to 30% of xylan polymer. Xylan is the main polysaccharide found in hemicelluloses which is a polysaccharide material hydrolyzed mainly by different xylanase enzymes (Singh *et al.*, 2009). The strategies of Complete degradation of xylan need the activity of endo-1,4- $\beta$ -xylanase (EC 3.2.1.8) and xylan 1,4- $\beta$ -xylosidase (EC 3.2.1.37) (Leitão *et al.*, 2017).

For best biotechnological applications, low-cost substrates allow the minimum cost of enzyme production while high costs cause limitations. As all know, the high cost of enzymes in ethanol production significantly affect their use in biofuel production (Klein-Marcuschamer *et al.*, 2012).

Using cheap agricultural wastes like lignocellulosic material for enzyme production are required to decrease the enzyme cost. such enzymes can use in the textile industry, foods, detergents, and production of bioethanol (Singhania et al., 2010, Klein- Marcuschamer et al., 2012). The most successful and economically excellent solutions to the problems created by non-renewable energy are the production and use of biofuels from which bioethanol is the most effective due to the easy production and good management. Annually, more than 16 billion gallons of bioethanol have been produced and production may be increased during the next ten years (Iram et al., 2020). Corn grains and sugarcane are used in the USA and Brazil to produce more than 85% of the world's bioethanol by converting dry milling corn starch to simple sugars which can be fermented to ethanol and carbon dioxide by the action of hydrolytic enzymes of yeast. However, only one-third of the nutritional components of these grains have used by the yeast cells and converted to ethanol while the other two parts remained as wastes called distillers dried grains with soluble (DDGS which had protein 25%, fat 10.8%, fiber 35%), and acid detergent fiber 15.1% (Liu and Rosentrater, 2016). Lignocellulosic products can be used for bioethanol production and replacing important grains that are the high energy source and used to feed humans and animals (Hsu et al., 2011). Cellulosic bioproducts can use to produce bioethanol through the pretreatment of bioproducts which changes it to fermentable reducing sugar by acid or enzyme hydrolysis then changing sugars to ethanol (Kumar et al., 2008). Acid hydrolysis is common but produces dangerous wastes (Sukumaran et al., 2009) while enzymatic hydrolysis has mild reaction conditions, good production of pure sugar, and no toxic wastes observed (Hamzah et al., 2011).

Using hydrolysis enzyme by microbial fermentation had low cost and high sugar yield from lignocellulosic biomass, which is significant for industrial applications (Maki *et al.*, 2009). Similarly, cellulase and hemicellulase are two important enzymes used to efficiently hydrolyze the abundant lignocellulosic materials, cross-linked in the plant structure with cellulose and hemicellulose (Seo *et al.*, 2014).

#### II. Conclusion

Xylan is a complex polysaccharide that is considered the main product of hemicelluloses. The xylanolytic enzymes which highly produced from bacteria and fungi and used mainly for the degradation of xylan are  $\beta$ -1,4-endoxylanase,  $\beta$ -xylosidase,  $\alpha$ -glucuronidase,  $\alpha$ -L-arabinofuranosidase, acetyl xylan esterase, and phenolic acid esterase. The xylanolytic enzyme had respected applications in different industrial fields. For example, paper industry, biofuels and surfactants enhancement, animal feed development, food and beverages, and hemicelluloses waste degradations.

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