A Review on Microbial Xylanases and Their Industrial Applications

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ARTICLE INFORMATION

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ABSTRACT

Xylan is most abundantly present polysaccharide after cellulose grouped as hemicelluloses. It is composed of D-xylopyranose residues. These polymers of hemicelluloses are broken by the enzymes called as xylanases.

Keywords: TBI, CT, CNS

Original Research Article

Introduction:

After cellulose the abundantly found polysaccharides is xylan (1). It is hemicellulose and in the secondary cell wall of lignified tissues of cereal, woody plant xylan is present (2). During the growth period also present in the primary cell wall and seeds and bulbs. Here it has a reserve function. In the case of hardwood, dry weight of plant cell wall have xylan almost 15-30 % while in the case of softwoods it reduces and only 7-12 % dry weight have xylan (3). Xylans have a vital role in the retaining integrity of cell wall with other like lignin, pectin, hemicellulose and cellulose. The cellulose microfibrils are protected from degradation by microorganism when xylan and lignin action are combined (3).

The structure of xylan is a homopolymer of D-xylopyranose residues. The monores ranges from 150-200 and polymerization occur and beta 1-4 linkages are found in structure. The backbone also has ferulic acid and glucuronic acid as a side groups. On the basis of these groups, xylanase are classified in different xylans such as glucuronoarabinoxylan, arabinoxylan, glucuronoxylan and homoxylan. The backbone of xylan when bound side group of uronic acid and arabinofuranose termed as glucuronoarabinoxylan. While in the case of arabinoxylan xylan backbone have 1-4 beta linkage and also with side group of a-arabinosyl. The main chain with beta 1-4 linked D-xylopyranosyl are substituted with one alpha 1-2 linkage 4-O-Methyl-D-glucuronic acid than xylan termed as glucuronoxylan (4).

The structure of 4-O-methylglucuronic acid display in the figure 1. When only xyloses units are bound may be in branched and linear form are homoxylan (5). The reactivity of xylan with hemicellulosic components, physical conformation and solubility depends on the side chain with the main chain of xylan. The extent and mode of enzyme action also determined by these side chain. When the side chain and groups linked with the hydroxyl groups of main chain xylan responsible for the complexity and heterogenisity of xylan.

The xylanolytic system contain β-xylosidase, acetylxylan esterase, glucuronidase, endo1,4-β-xylanases, p-coumaric acid esterase, ferulic acid esterase, α-arabinofuranosidase (6). On the basis of different studies xylanases found in many isoforms. The pre and post translation modification such as partial proteolysis or diferences in the degree of amidation and glycosylation and diferential mRNA processing are involved in the occurrence of these isoforms. In the hemicellulose some enzymes are present which degrade the xylan are termed as xylanases. As xylan have complex structure so in their hydrolysis xylanases are involved.The fungi, protozoans, snails, insects, marine algae, yeast, bacteria and crustaceans produced the xylanases enzymes. But mammals are not able to produce the xylanases. The commercial sources of xylan are filamentous fungi.

Objective:

The enzyme is abundantly produced by fungi, protozoans, snails, insects, marine algae, yeast, bacteria and crustaceans. Apart from the source of origin, microorganisms are promising candidates as a source for xylanase production. The bacterial xylanases have cellulose activity and also thermostable. The industrial methods used in production of food products, healthcare products and fuels usually produce toxic substances which can harm the environment. As a replacement, xylanases can play a major role to eradicate this environmental pollution. So, the purpose of this study is to review the industrial and biotechnological applications of xylanases.

Conclusion:

Xylanases produced for various industries are valuable in bioethanol production, animal nutrition, and wood pulp bio-

bleaching. For commercial production of xylanases, microorganisms are considered as one of the best sources because of their diversity in habitats and species.

Sources of Xylanases

In the nature xylanases are present abundantly. Xylanases are present in both eukaryotes and prokaryotes. The xylanases found in marine algae, protozoa, crustaceans, rumen bacteria, marine, terrestrial bacteria, fungi, snails, insects, germinating seeds and seeds of terrestrial plants (7). In the case of prokaryotes, cyanobacteria from marine environments and bacteria produced xylanases (8). The xylanases which present in plants are known as endoxylanases. The higher animals such as mollusk and Japanese pear fruits during over maturing period also produce xylanases. Some xylanases are also isolated and purified from different sources such as germinating barley, anaerobic bacterium Clostridium acetobutylicum and immature cucumber seeds (9).

Xyalanases isoforms

Endo -1,4-β-xylanases

The enzymes break the glycosidic bonds of xylan main chain and also decrease the polymerization of substrate, are Endo 1-4 beta xylanase. Xylan are not randomly attacked because hydrolysis depend on the substrate. The degree of branching, the presence of substituents and also length of main chain determined the action of enzymes. On the basis of end products of hydrolysis, endoxylanases are differentiated in different groups. The xylanases are may be de-branches and non-debranches. The de-branches enzymes are those which release the arabinose molecules while the non-debranching enzymes are those which do not release the arabinose. The bacterial and fungal xylanases are single subunit protein and most of them are glycosylated. The molecular weight of these subunits protein ranges from 8.5 to 85 kDa and pI values between 4.0 to 10.3 (10).

β- Xylosidases

On the basis of relative affinities for xylobiose and xylooligosaccharides, D-xylosidases are classified in different classes. These enzymes may be tetrameric, dimeric and even monomeric and also ranges molecular weight from 26 to 360kDa (Octavio et al., 2006). When these xylanases are purified than do not able to hydrolysis the xylan. These enzymes have great ability for polymerization of xylobiose while their affinity to polymerized the xylooligosaccharides is inversely proportional to xylobiose. The p-nitrophenyland o-nitrophenyl-β-D-xylopyranoside are artificial substrate and □-xylosidases are also able to hydrolysis these substrates. When xylan is catalyzed by several enzymes than β-xylosidases hydrolyzed the xylan.

α – Glucuronidases

The α-1, 2 linkages which present between glucuronic acid residues and β-D xylopyranosyl backbone units found in glucuronoxylan are hydrolyzed by these α- Glucuronidase.

α –Arabinofuranosidases

In the β-D-xylopyranosyl at position 2 and 3 L-arabinose removed by the enzymes Arabinofuranosidases. On the basis of the action of these enzymes classified as endo-1, 5-α-L-arabinase and exo-α-L-arabinofuranosidase. When only linear arabinans in the main chain are hydrolyzed than these enzymes are endo-1, 5-α-L-arabinase. On the hand when p-nitrophenyl-α- L-arabinofuranosides and branched arabinans are hydrolyzed than those enzymes are exo-α-L-arabinofuranosidase.

Acetylxylan esterase

In the acetylated xylans these xylanases at second and third position of xyloses residuces released the O-acetyl substituents. These xylanases also involved in the breakdown of xylan. During the hydrolysis of xylan the acetyl groups interfere with enzymes which hydrolyze the backbone by steric hindrance. Acetylxylan plays an important role in the hydrolysis of xylan, since the acetyl side-groups can interfere. The removal of acetyl groups facilitates the action of endoxylanases (11, 12).

Thermophilic xylanases

Sources of thermophilic xylanases :

Commom sources of xylanases are plants, seeds, insects, crustaceans, and microorganisms (12, 13). Xylanases produced from various industries are valuable in bioethanol production, animal nutrition, and wood pulp bio-bleaching. For commercial scale xylanase, microorganisms are regarded as one of the best sources because of their diversity in habitats and species. There are three potential sources of microbial xylanases that includes bacteria, archaea and fungi.

Bacterial sources

Thermophilic bacteria have been isolated from a variety of environments that produce xylanases. Recently, researchers isolated thermophilic Bacillus strains from Tunisian hot springs producing xylanases. Thermostable extracellular xylanase (XYN35) produced by the thermophilic anaerobic bacterium Caldicoprobacter algeriensis TH7C1(T) which was identified in hydrothermal hot springs. The Yunnan Province of China has hot springs in Baoshan where xylanase producing Gram-positive strain Rxl from the genus Thermoanaerobacterium was found. This Rx1 strain can be successfully grown using starch, xylan, polysaccharides and monosaccharides (14). A gram-positive actinomycete, Acidothermus cellulolyticus 11B, has heatstable xylan degrading enzyme Xyn10A was isolated from acidic hot springs in Yellowstone National Park hydrothermal hot springs and showed activity between 60°C and 100°C on birchwood xylans and oat spelt. From hot spring of New Zealand, xylanase (XYNB) producing extremely thermophilic bacterium Dictyoglomus thermophilum Rt46B.1 was isolated. This xylanase producing bacteria showed maximum growth at 70°C and it is a good source of thermophilic bacterial xylanase.

Archaeal sources

Researchers working in the field of biotechnology have discovered potential thermophilic archaea with the capability to produce enzymes which are stable under high salt concentrations, pH and extreme high temperatures (80–115°C). Thermophilic lignocelluloytic enzymes have been cloned, expressed and thus characterized in various expression systems. A thermoacidophile Sulfolobus solfataricus, capable of surviving in acidic volcanic locations and hot springs, was identified. This archaeon was capable to grow at extremely high temperature and extremely acidic pH i.e. 87°C and 2-4 respectively. Complex polysaccharides can be degraded by the carbohydrate degradative enzymes of

this archaeon such as endoglucanases and xylanases. There are three open reading frames (Sso1949, Sso1354, Sso2534) that encodes extracellular endoglucanases. These endoglucanases are in clan C and GH12 family.

Fungal sources

From last five decades, fungi are powerful producers of industrial enzymes. Filamentous fungi are exuberant producers of xylanases (13). The complex components present in the cell walls are broken down by a diversity of enzymes encoded by the genomes of following lignocellulolytic fungi that includes Phialophora sp. G5, Myceliophthora thermophile, Aspergillus fumigatus and Malbranchea pulchella. The Myceliophthora belongs to the class ascomycete and it was first reported as a thermophilic filamentous fungus by Apnis. Recent technologies for enzyme application in biomass derived fuels have used this potent cellulolytic organism. M. thermophila has distinctive attributes among xylanase producers. For example, it has a comparatively large number of arabino-xylanolytic and enzymes with degradative potential for lignocellulolsic structures. Actually, M. thermophila produces cellulolytic enzymes for its complete degradation. It has been disclosed by the analysis of genome sequence of M. thermophilia that a large range of genes is present in it which encodes heat-stable lignocellulolytic enzymes like lipases, proteases, carbohydrate-active enzymes, oxidoreductases and xylanases. Its genome has seven chromosomes with approximately 9110 genes located on them. Out of 9110 genes, CAZy enzymes are encoded by almost 250 genes. Out of these 250 genes, 180 are those which catalyzes the hydrolysis of glycosidic bonds while 13 are potential xylanases. An extensive range of cellulases and hemicellulases is produced by the genus Humicola that includes nontoxic and nonpathogenic both type of fungi (15). Thermophilic xylanolytic enzymes such as GH10 & GH11 xylanases are produced by a thermophilic fungus Humicola insolens Y1. Heatstable xylanases and cellulases are also produced by an isolated fungus named Thermoascus aurantiacus from the University of New Delhi, Aravali forest (15). Antioxidant compounds are also produced as a byproduct from it which could be used during the production of biofuel.

Applications of thermophilic xylanases

As xylanases have biotechnological potential especially for commercial applications so they are extensively produced from microorganisms. With the exception of specialized microorganisms, most living organisms can't withstand extremely hot environmental conditions. These specialized microorganisms are isolated from extreme environments and can tolerate within extreme acidic and alkaline pH, high salt concentrations and extremely low or high temperature. Xylanases have wide-ranging industrial applications because they do not denature under harsh industrial processing conditions due to their unique extremophilic properties. In addition, there are some other benefits such as: Low viscosity, high solubility of substrates, improved transfer rates and reduced contamination risks. Furthermore, the cost of these biocatalysts is reduced due to extension in the effective residence time of industrial enzymes during their processing. While residence time is extended due to thermostability of industrial enzymes.

In paper industry, bio-bleaching of pulp is done by using various thermophilic xylanases that can withstand alkaline pH and high temperatures. The removal of cellulose degrading enzymes is carried out by xylanase treatment of pulp. Chemical bleaching is replaced by bio-bleaching because it has reduced the toxic effects of effluents and the use of bleaching chemical. Moreover, it has improved the quality of fiber and pulp. There are some commercial xylanases available which are used in industries to improve the whiteness of pulp such as VAI-Xylanase, Pulpzyme HA and Novozyme 473. A xylanase was isolated from C. cellulans CKMX1 gained the enhanced whiteness over a wide range of pH with high temperature stability i.e., 60°C. Bacillus pumilus HBP8 produces a heat-stable xylanase xynHB that improved the whiteness of paper products in bleaching process and hence tensile strength and the burst factor of paper also improved. There are various microorganisms like Cellulosimicrobium spp. which possess suitable attributes regarding pulp bio-bleaching due to production of xylanolytic enzymes with negligible cellulose degradative action. This xylanolytic enzyme from C. cellulans CKMX1 showed optimum activity at alkaline pH and high temperature (16).

During the production of biochemicals and biofuels, biotechnological potential of xylanases make them valuable. A mild pretreatment of xylanases is very useful and trendy in industries to produce biomass with higher hemicellulosic content and to decrease the cost of second generation ethanol. For degradation of lignocellulosic biomass, Xylanases are also very useful in combination with other hydrolases such as laccases and cellulases during the production of biochemicals and biofuel. During the production of fermentable products by deconstruction of lignocellulosic material, microbial xylanases are very useful due to their robust thermostability which enables them to withstand harsh processing conditions. During the saccharification process, enzyme load is reduced very much by using xylanase due to its association with cellulases.

Industrial applications of xylanases

The bacterial xylanases have cellulose activity and also thermostable. Because of these properties xylanases have many applications in the industrial areas. Not only in the industrial application xylanases are also applied at commercial scale.

Baking industry

As wheat is the essential material in the baking. In the wheat hemicellulose (arabinoxylan) are present and cause serious restriction in the manufacturing of dough with good quality. The xylanases have ability to solubilize the water unextractable arabinoxylan as compared to those which is extractable arabinoxylan in water. Because of solubilizing ability as solubilized arabinoxylan performed better in manufacturing of bread. The bacterial xylanases sepecifically enhanced the dough viscosity and also on gluten agglomeration has negative effect. The xylanases have ability to convert hemicellulose from insoluble to soluble form.

Paper and pulp industry

As xylanases have ability to retain their activity at alkaline pH and high temperature, therefore are used in the biobleaching of pulps. Theses xylanases must free from cellulolytic enzymes to reserve the cellulose fibres (17) . Because cellulase enzymes have serious impacts in the degrading the quality of pulp, cellulose loss and also increase the cost for the treatment of effluent. From different microbes xylanases are isolated because of their competence and efficiency in the wood pulp bleaching (17). Most of bacterial xylanases have ability to unite with insoluble xylan and also with cellulose but still show activity only on the xylan. When bacterial xylanases ability compare with the fungal xylanases then the second one mean fungal sources xylanases have less ability to eliminate the hemicellulose. Fungal xylanases also responsible for the substantial kappa reduction. For the carbohydrates binding domain, supposed that fungal xylanases does not show the similar level of tenacity, also observed that fungal xylanases are less violent. When xylanases are used in the bleaching of pulp, decreases the quantity of Cl in the bleaching process. The xylanases are used in the biobleaching of eucalyptus kraft and not also increased the whiteness and brightness of pulp but also decreased the deplection of chlorin dioxide 10% and chlorine by 20%. In the prebleaching of eucalyptus krafts pulp similar outcomes are obtained.

Pharmaceutical industry

When the xylan are hydrolyze than xylooligosaccharides are formed, oligosaccharides are contain xylose subunits which bind with each other by beta 1-4 linkages. On the basis of presence of these xylose unites, are categories as xylotetrose, xylotriose and xylobiose . In the food application xylobiose used as prebiotics. By using the enzymatic and chemical method separately or both methods xylooligosaccharides are manufactured from xylan which contain the lignocellulosic biomass. When xylose are synthesized in high amount and have inhibitory effects on the production of xylooligosaccharides , the complex enzymes should have low activity of b-xylosidase and exoxylanase (17). Because of health promoting properties, xylooligosaccharides are very valuable product and commercially available in the market. The Rhodothermus, Bacillus, Thermobifida, Streptomyces and others are used in the enzymatic hydrolysis of xylan.

Textile industry

In the fabrics processing and desizing three main steps are involved (18). The first step involved the elimination of adhesive material while enhanced the whiteness of textile material and absorbency of bleaching included in the second step. On the third step is imparting of the typical whiteness of the fabric. The sizing material such as waxes and starch are used to increase the strength of fabric when weaving is produced by the mechanical abrasion. To make the fabric accessible for the successive processing steps, known as desizing, the adhesive wrapped material has to be removed. The desizing occurs by using strong alkaline oxidizing agents and high temperature. The next step is scouring which include the wetting, dyeing and also made the fabric free from material which inhibit the efficient finishing. Typically the scouring process is non-optimal and chemical intensive precess and also very expensive process because a large amount of water and energy used.

The hydrogen peroxide (H2O2)/sodium hypochlorite (bleaching) solutions and concentrated sodium hydroxide (scouring) solutions are harsh chemical basically used in the removal of inhibitory non-cellulosic impurities of pectic substances (0.4-1.2%), lignin containing proteins (1.0-1.9%), ashes (0.7-1.6%) and wax (0.4-1.2%) are present in the cuticle and also in the primary cell wall. The solution with high pH, used in the scouring and also produces different kind of threads to the environment safety. These solutions also attack the fiber cellulose non-specifically and as a result decreased the quality of fabric. The overflow of the textile industry suddenly increases the COD (chemical oxygen demand) and BOD (biological oxygen demand) values of water bodies. After different research these harsh chemical are exchanged with the commercial enzymes because these enzymes performed better to maintain the fabric standard properties and specially attack only on the non-cellulosic impurities as their target. When enzymatic systems are used than enzymes degrade the primary cell wall complex impurities to increase the water absorbing properties.

But still some cases are studied in which for scouring and resizing xylanases are used as compared to pectinases and cellulases are mostly used in the bioprocessing of fabric. But there are some problems when using enzymes in the processing of fabric. The major problem is the fragment of seed coats which bound with linters and fibers. So first the material is treated with xylanases enzymes to partially breakdown of the seed coat.

Discussion

Xylanases are enzymes that are produced by various prokaryotes and eukaryotes. These xylanases can be produced from their source of origin and can be exploited for human benefits. A variety of industrial processes like bioethanol production, animal feed, and bleaching of wood pulp use xylanases. Various microorganisms like Bacillus sp., Thermoanaerobacterium, Acidothermus cellulolyticus, and Dictyoglomus thermophilum are also involved in the production of xylanases at industrial level (15). With the help of these enzymes, the processes have become environment friendly. The toxic substances that are usually produced by industrial process are minimized. These enzymes are also used in combination with others for the production of bio-fuels. Such products can play a vital role in the maintenance of environment and ecosystem. Food industries are also making extensive use of this microbial product for production of good-quality foods. Apart from food and energy, pharmaceutical industries are also using enzymes for various health products (18). Therefore, xylanases are potential candidates as a replacement of various industrial chemicals to make all the industrial processes toxin free.

Conclusion

Xylanases are useful in various industries like bioethanol production, paper and pulp industry, animal feed, textile industry, etc. Thermophilic xylanases are particularly important because they can withstand harsh processing conditions. Recently, researchers are interested to isolate

more microbial species from different areas for their potential applications in various industries. Consequently, it can be rightly said that xylanases have great potential to be used in diverse industries to eliminate the toxic effects and create a better environment to live.

Authors Contribution

Tehreem Mujtaba came up with the idea and drafted the manuscript. Hareem Mohsin, as a supervisor, revised and made necessary changes in the manuscript.

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