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Microbial xylanase production and exploring it's potential applications

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ABSTRACT

This review delves into the fascinating realm of xylan, the primary component of hemicellulose, which ranks among the most abundant natural fibers worldwide. The complex and diverse structure of xylan poses a significant challenge for its breakdown. However, xylanases, extraordinary enzymes, possess the remarkable ability to cleave the intricate -1,4-glycosidic bond within this heterogeneous xylan structure. These exceptional catalysts are produced abundantly by a wide range of microorganisms, including bacteria, fungi, yeast, and marine algae, showcasing the vast biological sources of xylanases. By harnessing genetic regulation in enzyme biosynthesis, various strategies have been developed to scale up xylanase production. The escalating global demand for xylanases stems from their versatility in diverse industries. Their suitability for applications in food and feed, paper and pulp, textiles, medicines, and lignocellulosic biorefineries has rendered them highly sought-after. Consequently, in-depth analysis of complex xylan structures, microbial synthesis of xylanases, and exploration of potential industrial uses have become major areas of research. This comprehensive review provides a profound exploration of xylan and xylanases, shedding light on their remarkable properties, production methods, and wide-ranging applications. The insights presented here establish a foundation for further advancements and the utilization of xylanases across various industries.

Keywords: Xylanase, hemicellulose, xylan, submerge fermentation, solid state fermentation.

INTRODUCTION

The xylanases (E.C. 3.2.1.8, 1,4-D xylan-xylan hydrolase) break down xylan, a substance found in plant polysaccharides. Xylan is a complex polysaccharide with a backbone of xylose residues and 1,4-glycosidic bonds connecting each subunit. D-arabinofuranoside or D-glucuronic acid are useful for creating branches off the xylan backbone. The backbone of the complex polysaccharide xylan is composed of xylose residues joined by 1,4-glycosidic linkages (Whistler and Richards 1970).

The 1,4-linked 1,4-D-xylopyranosyl Units found in feruloyl, acetyl, -L-arabinofuranosyl, and -D-glucuronyl residues make up hemicellulose, the second most common polysaccharide after cellulose (Grange et al., 2001).

Xylan is one of the most widely distributed polysaccharides in nature. About one-third of the dry weight of higher plants is made up of xylan. It has side branches made of various substituent groups and a linear backbone made of 1, 4 linked xylose residues. It is a complex heteropolysaccharide. Xylan, which serves as an interface between lignin and other polysaccharides, is a component of the secondary cell wall (Dhiman et al., 2008). The current trend towards a complex and more effective utilisation of biomass has drawn a lot of attention to the exploitation of xylans as biopolymer resources in recent years. Xylans are present in quite significant amounts in organic materials. agricultural by-products and waste from regenerating forests, such as wood shavings, hulls, cobs, stems, and so forth. They can be easily recovered from biomass. The study of algal xylenes in biopolymer research is currently expanding. However, the Xylans' potential hasn't been fully realised. Due to the wide variety of structures present in even a single plant, it is difficult to use xylans individually (Stephen, 1983; Neto et al., 1997). Xylanases are produced by a variety of living things, including microorganisms, protozoans, and mollusks. They can also be found in higher animals' rumens (Beg et al., 2001). The xylanases are produced in significant amounts by microorganisms like bacteria, fungi, and actinomycetes (Motta et al., 2013). The commercial use of xylanase in many industrial processes has been constrained because of a number of issues. These include physical barriers that prevent xylanase enzymes from accessing the substrate, the limited hydrolysis potential of xylans due to their diverged branched structure, a narrow pH range, thermal instability, end product inhibition, and the high cost of enzyme production. By using cheap substrates and the solid-state fermentation (SSF) method, the final two difficulties can be somewhat overcome (Walia et al., 2013).

The use of lignocellulosic biomass (LCB) for producing different biochemicals like bioethanol, enzymes, and value-added compounds has significantly advanced in recent years. The opportunity to examine xylanase's hydrolytic ability for efficient saccharification of LCB for the production of ethanol and xylooligosaccharides results from this. Additional uses for xylanase include the bleaching of pulp and paper, food, feed, and pharmaceuticals. In the current review, various features of the xylanase-producing microbial strain are discussed. As the demand for xylanolytic enzymes increases across a range of industrial sectors, so does our understanding of xylan and xylanases. This review makes an effort to concentrate on the production, consumption, and industrial uses of enzymes in this context.

Structure and Classification of Xylan

Hemicellulose, a highly abundant macromolecule, plays a vital role in various biological systems across the globe. It constitutes a significant portion, ranging from 20% to 30%, of the dry weight of wood. In conjunction with cellulose and lignin, hemicellulose serves as a fundamental structural component within plant cell walls and lignocellulosic materials. Comprised of distinct monomer units, hemicellulose encompasses pentoses (carbohydrates with five carbon atoms), hexoses (carbohydrates with six carbon atoms), and acid sugars. Among the hexose units, galactose, glucose, and mannose are the primary constituents. In terms of acid sugars, the principal components are 4-O-methylglucuronic acid and galacturonic acid. Additionally, the primary pentose units within hemicellulose are arabinose and xylose. This intricate composition highlights the diverse nature of hemicellulose, contributing to its essential functions in the structural integrity and functionality of plant cells and lingo-cellulosic materials (Pettersen, 1984). In the context of plant biology, hemicellulose plays a crucial role in the arrangement and organization of the major components of plant cell walls, namely lignin and cellulose. Within plant tissues, the presence of hemicelluloses, specifically xylans, contributes to the separation and distinction between lignin and the group of cellulose fibers. Xylans, a type of hemicellulose, exhibit a structural chemistry that is compatible with their intended function. They possess side group substitutions that enable the formation of a protective covering around the underlying cellulose strands through hydrogen bonding interactions. This covering, facilitated by the hemicellulose matrix, helps to shield the cellulose fibers from external factors and provides structural support to the overall plant cell wall. The intricate network of hydrogen bonds between the xylan molecules and the cellulose fibers aids in

maintaining the structural integrity of the cell wall, ensuring its resilience and stability. By forming this interlinking structure, hemicelluloses contribute to the overall strength and rigidity of plant tissues, enabling them to withstand various mechanical stresses and environmental challenges (Joseleau *et al*., 1992).

Xylan, a crucial component of plant tissues, exhibits structural variations that can be classified into several distinct forms. These divisions include homoxylans, glucuronoxylan, arabinoglucuronoxylan, glucurono-arabinoxylan, arabinoxylan, and heteroxylan. Homoxylans form the basic structure of xylan, characterized by a backbone primarily composed of xylose units. Glucuronoxylan incorporates xylose units with glucuronic acid side groups, enhancing its interaction with other components of the plant cell wall. Arabinoglucuronoxylan combines arabinose and glucuronic acid side groups with xylose units, contributing to structural diversity and flexibility. Glucuronoarabinoxylan contains xylose units linked to glucuronic acid and arabinose side groups, adding complexity and functionality to the xylan structure. Arabinoxylan predominantly consists of xylose units linked to arabinose side groups, commonly found in cereal grains and contributing to their dietary fiber content. Heteroxylan encompasses various side group combinations, such as acetyl and ferulic acid substitutions, imparting unique properties and functions to xylan. The division of xylan into these subtypes reflects the diverse composition and structural variations of hemicellulose in plant cell walls. Each form of xylan contributes to the overall strength, flexibility, and interactions within the plant tissue. Understanding the different xylan subtypes provides insights into the intricate nature of plant cell wall architecture and its role in plant growth and development (Brandt et al., 2013).

Homoxylan

In homoxylan form, xylan consists only of xylose residues and lacks any other monosaccharides. The structure of homoxylan can vary based on the specific types of glycosidic linkages present between the xylose residues. According to a study by Ebringerova and Heinze in 2000, homoxylan with β-(1-3) glycosidic linkages has been reported to be found in green algae called Caulerpa sp. This means that the xylose residues in this form of xylan are connected to each other through β-(1-3) glycosidic bonds. Homoxylan with the structure of β-(1-3 and 1-4 linkages) has been reported to be found in two orders of red algae, namely Palmariales and Nemaliales. This indicates that in this form of xylan, the xylose residues are linked together through a combination of β-(1-3) and β-(1-4) glycosidic bonds. These findings suggest that different plant species and algae can have variations in the structure of xylan, with varying types of glycosidic linkages between the xylose residues. The presence of xylan with different linkages in these organisms highlights the diversity of polysaccharide structures in the plant kingdom and provides insights into the evolution and adaptation of plant cell walls (Zhou X et al., 2017). The analysis reveals that β-1,3-linkages are evenly distributed in the cell wall in the form of pentamers, which are five-unit chains (Ebringerova et al., 2005).

Arabinoxylan

Arabinoxylans, which belong to the group of hemicelluloses, are predominantly located in the outer layer and endosperm cell walls of cereal plants. These arabinoxylans are connected or linked to cellulose and lignin, two other components of the cell wall. Arabinoxylans play a crucial role in providing structural support and integrity to the cell wall of cereals (Izydorczyk and Biliaderis, 1995).

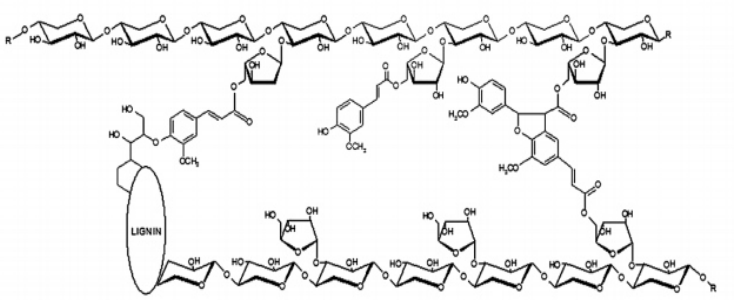

Fig. 1 Structure of arabinoxylan (Döring et al., 2016)

Arabinoxylan is a major component of cellulose, a key structural component found in many plants. It constitutes a significant proportion of the cellulose content in various plant species. Arabinoxylan plays a vital role in binding and connecting different parts of the cell wall, contributing to the overall strength and integrity of the cell wall. It helps to reinforce the structure and provide support to the plant cells (Scheller and Ulvskov, 2010). (1-4) glycosidic linkages connect the arabinoxylan's linear chain of -D-xylopyranosyl. Izydorczyk (2008) found that monomeric -L-arabinofuranoside residues were attached to some of the xylopyranosyl chains at the O-3, O-4, and/or both O-2,3 positions.

The same study claims that based on the arabinofuranoside position and the number of substitutions, four primary structural units can be distinguished: monosubstituted xylopyranosyl at O-2 or O-3, disubstituted xylopyranosyl at both O-3,4 and unsubstituted xylopyranosyl (Izydorczyk & Dexter, 2008). Some researchers claim that the outer layers of cereal grains like wheat, corn, rice, barley, oat, rye and sorghum contain arabinoxylan. (Fincher and Stone, 1986; Saeed et al., 2011; Vinkx & Delcour, 1996; Zhang et al., 2014). Other plant tissues, including those of bamboo (Ishii et al., 1990), ryegrass (Hartley & Jones, 1976), sugarcane bagasse (Sun et al., 2004), grape pomace (Minjares-Fuentes et al., 2016), and garlic straws (Kallel et al., 2015), have also been found to contain arabinoxylan.

Glucuronoxylan

According to Gordahl et al., 2003, the main hemicellulose component of hardwood, glucuronoxylan, makes up 15–30% of the dry material. The 1- 4 linked D-xylopyranosyl (DXylp) residues that make up glucuronoxylan's backbone are invariably replaced at position 2 by 4-O-methyl-D-glucopyranosyl uronic acid (4-O-MeGlcA). The polysaccharide framework is made up of about 200 DXylp residues. In some xylose units, position 2 only contains one terminal side chain of the glucuronic acid residue (Timell and T. E. 1967). Teleman et al. used dimethyl sulphoxide (DMSO) to extract hemicelluloses from birch and beech. By using NMR analysis, they were able to determine that the main ingredient is O-acetyl-(4-O-methylglucurono)-xylan, which has an average of one 4-O-MeGlcA substituent for every 15 D-xylose residues (Teleman et al., 2002).

Arabinoglucuronoxylan and glucuronoarabino-xylan: a key component of non-woody materials

Glucuronoarabinoxylan (GAX) is a major component found in non-woody materials. It is composed of a backbone made up of β– (1-4)-D-Xylp (xylose) units, which are substituted at positions C-2 or C-3 with MeGlcA (4-O-methylglucuronic acid) and α-L-arabinofuranosyl (α-L-Araf) units. GAX can also have a slight degree of acetylation, as suggested by research conducted by Peng et al. in 2012 (Peng F, et al., 2012). The structure of GAX, characterized by an AXE backbone, results in significantly fewer uronic acid side chains compared to α-L-Araf. In fact, GAX has approximately ten times fewer uronic acid side chains than α-L-Araf. Additionally, some Xylp residues within GAX can contain twice as many α-L-Araf units (Ebringerova et al., 2005). These findings were observed in a study by Shi et al. in 2013, where they analyzed hemicelluloses extracted from dendrocalamus using an alkali method. The study revealed that the majority of the soluble hemicelluloses present were GAX. GAX was identified as having a linear β-(1-4)-Xylp backbone, with α-L-Araf and/or 4-O-MeGlcA units replacing the Xylp units at positions 2 or 3 (Shi et al., 2013).

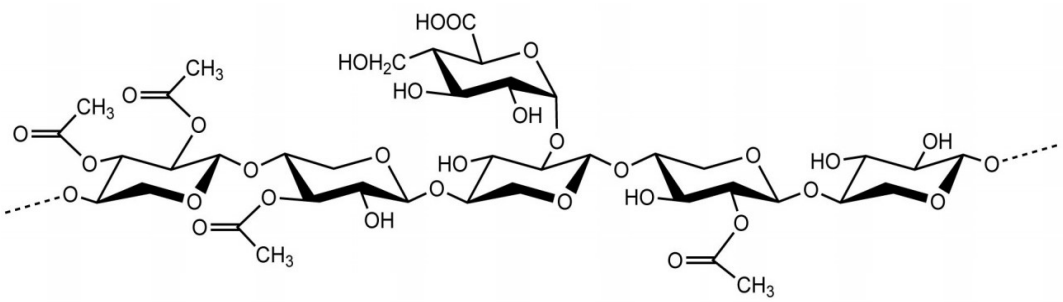

Fig. 2 Structure of Structure of glucuronoxylan (Hu et al., 2017).

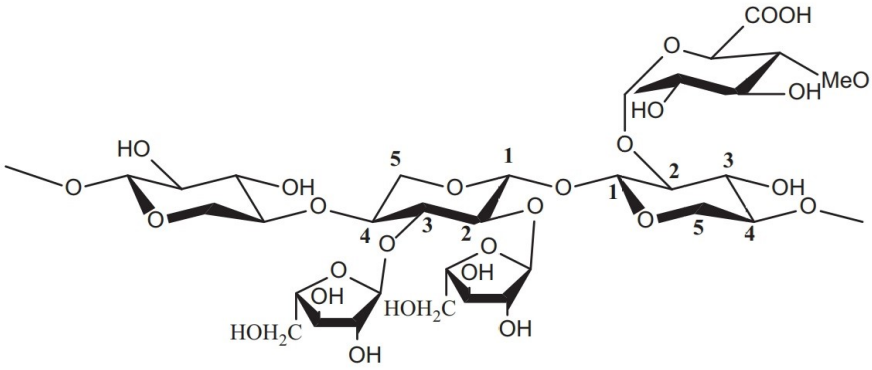

Fig. 3 Structure of glucuronoarabinoxylan (Fu G. et al., 2019)

Furthermore, other researchers, such as Yamasaki et al. in 2011, have successfully extracted arabinoglucuronoxylans from the hemicellulose of Sugi (Cryptomeria japonica) and Hinoki (Chamaecyparis obtusa), indicating the presence and importance of GAX in various non-woody materials (Yamasaki T, et al. 2011)." (Ebringerova A, et al. 2005). Shi et al.'s study on dendrocalamus hemicelluloses extracted with alkali. The majority of the soluble hemicelluloses according to neutral sugar analysis were GAX, which had linear β-(1-4)-Xylp backbone with α-L-Araf and/or 4-O-MeGlcA units replaced at the position 2 or 3 (Shi Z, et al. 2013). Some researchers extracted arabinoglucuronoxylans from the hemicellulose of Sugi (Cryptomeria japonica) and Hinoki (Chamaecyparis obtuse) (Yamasaki et al., 2011).

STRATAGIES TO CARRY OUT EXTTRACTION OF XYLAN

Corn hulls are commonly known as a source of xylan (Chaniaud et al., 1995). However, there are several other readily accessible sources of xylan that exist in large quantities. For example, sorghum stalks and sunflower hulls, which are byproducts of sunflower oil production, have been identified as potential sources of xylan (Bazaus et al., 1993). Xylan has also been isolated from various sources such as the husk of red grills (Swamy and Salimalh, 1990; Brlla et al., 1997), ramie fibers (Bhaduri et al., 1995), olive pulp (Coimbra et al., 1994), steamed bamboo grass (Aoyama and Seki, 1994), flax (Van Hazendonk et al., 1996), sisal (Stewart et al., 1997), and wheat straw (Sun et al., 1998).

Different methods are available for the extraction of xylan, including alkali extraction methods, organic solvent extraction, steam expulsion, hydrothermal methods, microwave irradiation, and ultrasonic treatment. In alkali extraction, the choice of alkali used in the process impacts the yield of hemicellulose, with NaOH and KOH being recommended for their effectiveness in obtaining high xylan yields. Among the two, KOH is superior in terms of yield, while NaOH-extracted fractions tend to have higher purity (Lawther et al., 1996; Curling et al., 2007; Sun et al., 1998; Xu et al., 2007).

In a study examining the extraction process of coconut, a two-step alkali extraction process was utilized. The coconut was first treated with 10% NaOH for 8 hours at 65°C to obtain xylan, which was then subjected to a second round of hydrothermal treatment. The first phase yielded approximately 40-52% of available xylan, with a total recovery exceeding 90% (reference not provided).

When using organic solvents for xylan extraction, the structure of xylan, including its acetyl groups, remains preserved. Organic solvents such as alcohols and organic acids are considered neutral solvents for xylan extraction due to their minimal alteration or degradation of the original structure (Maurya et al., 2015; Zhang et al., 2016). Additionally, a DMSO/LiCl system has been employed for extracting xylan from fruits and vegetables, resulting in relatively fine structures (Assor et al., 2013).

High-pressure steam treatment is a preferred method for lignocellulosic biomass due to its high efficiency. The steam explosion during the process not only breaks down the material rapidly but also hydrolyzes xylan, causing the destruction of the essential structure of plant material (Cara et al., 2006).

Microwave-assisted extraction is an alternative method to traditional extraction techniques, known for its distinct mechanism, low cost, high extraction rates, and selective product separation without significant molecular structure alteration. Some researchers have found that microwave treatment produces better results compared to conventional procedures, recovering up to 60% of xylan from birch wood despite some degradation (Panthapulakkal et al., 2015).

Ultrasonic irradiation has been shown to greatly enhance the extraction of polysaccharides, including low molecular weight compounds when separating plant material with solvents (Mason et al., 1966; Panchey et al., 1994). Ultrasonic treatment has also been applied to xylan, with no significant alteration to the structural and molecular characteristics of corn cob and corn shell xylan (Ebringerová et al., 1997).

INTRODUCTION OF XYALANASE ENZYME

Endo- and exo-xylanases are the two main enzymes involved in the hydrolysis of the xylan backbone. Some side chain cleaving enzymes, in addition to main chain cleaving enzymes, are necessary for the hydrolysis of xylan. Acetyl esterase, L-arabinofuranosidase, and D-glucuronidase are just a few of the side-chain cleaving enzymes that have been discovered to work together to hydrolyze xylan (Lee and Forsberg, 1987). There are mainly three methods for categorizing xylanases.

Based on molecular weight and pI

The classification of xylanases into high or low molecular weight or pI categories allows researchers to gain insights into the enzyme's properties and behaviour. High molecular weight xylanases generally have larger molecular sizes and can exhibit different substrate specificities and catalytic activities compared to low molecular weight xylanases. The availability of class information in enzyme purification and initial characterization is considered an attractive feature of this classification. By knowing the molecular weight or pI class of a xylanase, researchers can make informed decisions about the purification strategies, optimize reaction conditions, and better understand the enzyme's structure-function relationships (reference not provided). Overall, the classification of xylanases based on molecular weight or pI provides a useful framework for studying and characterizing these enzymes, aiding in their purification and initial characterization processes (Ebringerová et al., 1997).

Based on crystal structure

Xylanases, enzymes that degrade xylan, can be classified into two structural families: Family F, also known as Family 10, and Family G, also known as Family 11. This structural division provides insights into the characteristics and properties of xylanases based on their family classification (Buchert et al., 1995).

Family 11 xylanases are characterized by their lower molecular weight compared to Family 10 xylanases. Family 11 xylanases typically exhibit a compact structure and have a smaller size compared to Family 10 xylanases (Buchert et al., 1995). These enzymes are often active in a wide pH range and have been found in various microorganisms, including bacteria and fungi (Wong et al., 2014).

On the other hand, Family 10 xylanases are generally larger in size and have a higher molecular weight compared to Family 11 xylanases. They possess a more extended structure and are known for their versatility in hydrolyzing xylan from different sources (Buchert et al., 1995). Family 10 xylanases have been identified in various organisms, including bacteria, fungi, and plants, and they play important roles in the degradation of xylan in nature (Collins et al., 2005).

The structural division into Family F (Family 10) and Family G (Family 11) provides a useful framework for understanding the molecular characteristics and functional properties of xylanases. These families have distinct structural features and are associated with different enzyme sizes and activities. The classification aids in the characterization, classification, and utilization of xylanases in various industrial and biotechnological applications.

Based on enzyme kinetic

Based on the kinetic characteristics and substrate selectivity of xylanases, a third type of classification can be made. This classification provides insights into the enzymatic properties and behavior of xylanases (Subramaniyan and Prema, 2002).

Enzyme kinetic parameters such as substrate affinity (Km), turnover rate (kcat), and catalytic efficiency (kcat/Km) can vary among different xylanases. These kinetic characteristics influence the enzyme's substrate selectivity and efficiency in degrading xylan (Subramaniyan and Prema, 2002). Xylanases may exhibit varying degrees of substrate specificity towards different xylan substrates, depending on their kinetic properties.
The classification based on enzyme kinetics allows researchers to gain a deeper understanding of the enzymatic activity and substrate preference of xylanases. It provides valuable information for enzyme characterization, optimization of reaction conditions, and selection of suitable enzymes for specific applications in industries such as pulp and paper, biofuel production, and food processing (Beg et al., 2001).

The kinetic classification of xylanases contributes to the identification and characterization of enzymes with desirable properties for efficient xylan degradation. By considering the kinetic parameters and substrate specificity, researchers can better evaluate the potential of xylanases for specific industrial applications and tailor their use accordingly.

SOURCES OF XYLANASE

Xylanase is a widely distributed enzyme in nature, found in both prokaryotes and eukaryotes. It has been identified in various organisms, including rumen bacteria, snails, crustaceans, insects, terrestrial plant seeds, protozoa, fungi, and marine algae (chakdar et al., 2016; Walia et al., 2013; Yamaura et al., 1997). Prokaryotes, such as bacteria and cyanobacteria from maritime environments, are also known to produce xylanase (Annamalai et al., 2009).

Rumen bacteria, which are found in the digestive systems of ruminant animals, have been shown to produce xylanase. Studies by Chakdar et al. (2016) have highlighted the presence of xylanase in rumen bacteria, indicating its role in the breakdown of xylan-rich plant materials during digestion.

In addition, Yamaura et al. (1997) observed the occurrence of xylanase in snails and crustaceans. This suggests that these organisms utilize xylanase for the digestion of plant cell wall components, which often contain xylan.

Various insects, including beetles and termites, have also been found to produce xylanase. These insects rely on xylanase to degrade the complex polysaccharides present in their food sources, such as wood and plant fibers (Walia et al., 2013).
Furthermore, xylanase has been identified in terrestrial plant seeds, protozoa, fungi, and marine algae. These organisms produce xylanase to hydrolyze xylan, which is a major component of hemicellulose, and utilize the released sugars as an energy source (Walia et al., 2013).

Among prokaryotes, bacteria and cyanobacteria from maritime environments have been shown to produce xylanase. Annamalai et al. (2009) conducted studies on bacteria and cyanobacteria isolated from marine environments, highlighting their ability to produce xylanase enzymes for the breakdown of xylan-containing substrates.

The widespread occurrence of xylanase in various organisms indicates its importance in the degradation of xylan and utilization of plant cell wall components. These enzymes play crucial roles in the natural carbon cycle and have significant applications in various industrial sectors, including biofuel production, pulp and paper industry, and food processing.

Bacterial sources of xylanase

It is important to note that the bacterial source, molecular weight, optimum temperature, and pH of xylanase can provide valuable insights into its characteristics and potential applications in various industries, such as biofuel production, pulp and paper industry, and food processing. These parameters help in understanding the enzymatic activity and optimizing the conditions for its efficient utilization. It has been widely reported that *Bacillus* species, such as *B. halodurans*, *B. pumilus*, and *B. subtilis*, are the microorganisms that produce the strongest xylanolytic enzymes (Gupta et al., 2015; Thomas et al., 2014; Banka et al., 2014). The molecular weight of the obtained xylanase, as well as the ideal temperature and pH required for production, are all outlined in table 1 along with the bacterial sources.

Table: 1 Bacterial source, molecular weight of obtained xylanase along with its optimum temperature and pH

Bacteria	Molecular weight (kD) of xylanase	Optimum temperature	Optimum pH	Reference
Thermoanaerobacterium sp. JW/SL–YS 485	24-180	80	6.2	Shao et al. (1995)
Cellulomonas fimi	14-150	40-45	5-6.5	Khanna and Gauri (1993)
Geobacillus thermoleovorans	45-50	70	7-8	Gerasimova .and Kuisiene (2012)
Bacillus licheniformis	14.4-94	50	10	Kamble and Jadhav (2012)
Acidobacterium capsulatum	41	5	65	Inagaki et al. (1998)

Table: 2 Fungal source, molecular weight of obtained xylanase along with its optimum temperature and pH

Fungus	Molecular weight(kD)	Optimum temperature	Optimum pH	Reference
Aspergillus niger	13.5- 14	45	5.5	Frederick et al., 1985
Fusarium oxysporum	20.8- 23.5	60	6	Christakopoulos et al., 1996
Aspergillus sojae	32.7-35.5	50-60	5-5.5	Kimura et al., 1995
Trichoderma reesei	19-20	40-45	5-5.5	Tenkanen et al., 1992
Cephalosporium sp.	30, 70	40	8	Bansod et al., 1993

5.2 Fungal sources of xylanase

The most often used mesophilic fungus for commercial manufacture of xylanase is those belonging to the genera *Aspergillus* and *Trichoderma.* Table 2 lists the fungal sources, ideal temperature, and pH requirements for production along with the molecular weight of the obtained xylanase.

6. FERMENTATION STRATEGIES

6.1 Solid state fermentation

Solid-state fermentation (SSF) is the growth of microorganisms on moist substrates without the presence of freely flowing water. In comparison to liquid-batch fermentation, SSF processes require less liquid for product recovery, as well as cheap substrate, low cultivation costs, and lower contamination risks. Utilising widely accessible and inexpensive agricultural waste products, such as wheat bran, maize cobs, rice bran, rice husk, and other comparable substrates, to increase xylanase yields using SSF can lower the cost of producing bio-bleached paper. This has made implementing this environmentally friendly technology in the paper industry simpler. *Streptomyces* sp. QG-11-3 (Beg et al., 2000) uses wheat bran and eucalyptus Kraft pulp as the primary solid substrates, with substrate-to-moisture ratios of 1:2.5 and 1:3, respectively, to produce the highest levels of xylanase in solid-state fermentation. However, as the moisture level rose or fell, the xylanase yield margin shrunk. The most xylanase, however, was found to be produced by Bacillus sp. A-009 at a lower solid-to-substrate moisture ratio of 1:1 (Gessesse and Mamo 1999). When grown in SSF as opposed to submerged fermentation using wheat straw and sugarcane bagasse, the thermophilic *Melanocarpus albomyces* IIS-68 has been shown to produce more xylanase (Jain 1995). The SSF of Trichoderma Koenig was supplemented with corn cob and powdered pineapple peel, which led to an increase in xylanase production (Bandikari et al., 2014).

6.2 Submerge fermentation

Submerged fermentation (SmF) employing bacteria and fungi has been widely recognized as the most effective method for xylanase production (Polizeli et al., 2005; Bajpai, 2014). In fact, statistics indicate that SmF accounts for approximately 90% of global xylanase production. This method allows for the utilization of various xylan-degrading enzymes, which can lead to enhanced biomass utilization and increased synthesis of xylanase. SmF offers several advantages in the production of xylanase. Firstly, it provides a homogeneous condition throughout the fermentation medium, ensuring optimal growth and enzyme production (Guleria et al., 2013). Additionally, SmF is a well-characterized technique, with established protocols and parameters for effective production. It also offers ease of scaling up, allowing for large-scale production of xylanase to meet industrial demands.

However, SmF is not without its limitations for industrial applications. One of the major drawbacks is the high maintenance costs associated with the fermentation process. The requirements for energy and resources, such as aeration, stirring, and temperature control, can contribute significantly to the overall cost (Virupakshi et al., 2005). Furthermore, downstream processing of xylanase from the fermentation broth can be complex and costly, adding to the challenges of industrial implementation.

Despite these drawbacks, the advantages of SmF, such as its well-established protocols, scalability, and potential for improved biomass utilization, make it a preferred method for xylanase production. Researchers and industry professionals continue to explore ways to optimize SmF processes and address its limitations in order to enhance the efficiency and cost-effectiveness of xylanase production.

7. COMMERCIALLY AVAILABALE XYLANASES

Since the use of xylanase is gradually increasing, numerous commercial companies have appeared to conduct research and sell this extremely powerful enzyme, which is sold under various trade names (Table 3).

Table: 3 Manufacturer of xylanase

Supplier	Product name	Application
Sankyo, Japan	Sanzyme PX, Alpelase F	Feed, Food
Novo Nordisk, Denmark	Pulpzyme HA, Pulpzyme HB, Pulpzyme HC	Pulp bleaching
Bicon India, Bangalore	Bleachzyme F	Pulp bleaching
Enzyme Development, USA	Enzeko xylanase	Baking, food, feed
Rohm, Germany	Release 7118	Food
Alltech, USA	Allzym PT	Animal feed
Amano Pharmaceutical Co, Japan	Amano 90	Food, Feed, Pharmaceutical
Ciba Giegy, Switzerland	Sanzyme	Food and baking
Alko Rajamaki, Finland	Ecopulp	Food, Feed

8. APPLICATION OF XYLANASE

8.1 Paper pulp industry

In the pulp and paper industry, chemical bleaching is commonly employed to enhance the brightness of paper. However, this process can have detrimental effects on pulp output, pulp viscosity, and the integrity of cellulose components. Decreased viscosity is particularly undesirable as it is inversely related to cellulose polymerization and paper strength (Cheng et al., 2013).

To address these challenges, lignohemicellulolytic enzymes are increasingly utilized for bleaching purposes worldwide. Xylanases play a crucial role in the hydrolysis of xylan, which is linked to cellulose and lignin in wood fibers. Disruption of xylan leads to the separation of this component, resulting in increased swelling in the fiber wall and enhanced lignin extraction from the pulp (Thomas et al., 2015).

To further improve cellulose brightness, a combination of xylanase and lignin-degrading enzymes is often employed (Viikari et al., 1994). However, the focus of research studies has predominantly been on the impact of these enzymes on reducing chemical consumption rather than their effects on pulp yield and viscosity. Typically, xylanase is applied to the material prior to chemical bleaching. In the presence of xylanase, reprecipitated xylan is hydrolyzed, accelerating cellulose bleaching and reducing the need for excessive chemical usage. Consequently, this method contributes to the reduction of toxic substances released into the environment (Cheng et al., 2013).

Overall, the utilization of lignohemicellulolytic enzymes, particularly xylanase, in the bleaching process offers the potential to improve pulp quality, reduce chemical consumption, and minimize environmental impact.

8.2 Juice industry

Xylanases have found applications in various industries, including the clarification of must and juices, fruit and vegetable liquefaction, and enhancing fermentation efficiency in beer brewing.

When used in combination with cellulose and pectinase, xylanases aid in the clarification of must and juices by

liquefying fruits and vegetables (Beg et al., 2001). The enzymatic treatment, followed by centrifugation and filtration techniques, helps remove suspended and undissolved solids, reducing viscosity and preventing the formation of clusters. This process improves the purity, flavor, and color of the juice (Danalache et al., 2018).

In the clarification of tomato liquid, glutaraldehyde-activated immobilized xylanase has been employed. Additionally, xylanase from *P. acidilactici* GC25 has been used to treat various fruits including kiwi, apple, peach, orange, apricot, grape, and pomegranate. These treatments resulted in an increase in reducing sugar content and a decrease in juice turbidity (Adiguzel et al., 2019).

In the fermentation industry, xylanases are utilized to enhance process efficiency in sectors such as beer brewing. By reducing the viscosity of substrates containing arabinoxylans, such as wheat and barley, xylanases serve as a pre-treatment, facilitating improved fermentation outcomes (Subramaniyan & Prema, 2002).
Overall, the use of xylanases in combination with other enzymes contributes to the clarification and improvement of must and juices, liquefaction of fruits and vegetables, and enhanced fermentation efficiency in the beer brewing industry.

8.3 Animal feed industry

Xylanase enzymes are used in the pre-treatment of forage products to enhance ruminant feed digestibility and facilitate composting (Gilbert & Hazlewood, 1993). Xylanases play a crucial role in animal feed by reducing the viscosity of raw substances and breaking down the arabinoxylan present in feed ingredients.

In ruminant feed applications, the effectiveness of xylanases was demonstrated using Aspergillus japonicus CO_3, which exhibited high stability and excellent production of endoxylanase and cellulase in a goat ruminal environment (Facchini et al., 2011). The presence of this enzyme improved the digestibility of feed components.

Furthermore, the addition of xylanase to poultry feed has shown benefits such as increased apparent nitrogen and fiber absorption, as well as improved feed transit time. Studies have indicated that the inclusion of enzymes, including xylanase, in boiled castor seed meal (up to 150g/kg) was suitable and did not have any adverse effects on growth efficiency or blood constituents (Babalola et al., 2006).

It is important to note that complete eradication of xylan is not desired, as hemicelluloses, including xylan, are essential dietary components. Complete removal may exacerbate bowel diseases (Mandal, 2015), emphasizing the need for balanced enzyme supplementation in animal feed formulations.

Overall, the use of xylanase enzymes in the pretreatment of forage products contributes to improved digestibility in ruminant feed and facilitates composting. In both ruminant and poultry feed applications, the addition of xylanase enhances nutrient absorption and feed efficiency.

8.4 Textile industry

The use of xylanases in the recovery of textile fibers and paper fibers differs primarily in the accessibility of the fibers to the enzymes. Textile fibers obtained from plants, such as flux and rayon fibers, are extracted from soft stems that have lower lignin content compared to hardwoods and softwoods. This lower lignin content makes it easier for xylanases to reach and act on the fibers. For example, when dried ramie stems are incubated with xylanases, complete release of cellulosic fibers occurs, requiring less bleaching (Milagres & Prade, 1994).

Xylanases play a beneficial role in the separation of fibers from the interstices of plant cell walls in various processes, although the precise mechanisms by which they facilitate lignin removal are not yet fully understood (Paice & Jurasek, 1984; Viikari et al., 1990; Archibald, 1992; Paice et al., 1992). An added advantage of enzymatic treatment of intact cellulosic fibers is that it does not lead to the oxidation of lignin, reducing fiber discoloration. This enzymatic activity may contribute to the recovery of non-discolored cellulosic fibers (Milagres & Prade, 1994).
Despite the potential benefits and practicality of enzymatic preparation of textile fibers, this area has received relatively little attention in molecular biochemistry. However, it holds promise as a suitable platform for commercial biotechnological advancements and pilot-scale applications (Milagres & Prade, 1994).

8.5 Seed germination and fruit ripening process

Late in the germination process, seeds naturally produce xylanases, which play a role in facilitating nutrient accessibility for the new plant. These enzymes aid in the breakdown of polysaccharides, such as xylans, in the cell wall matrix of the seeds, allowing the plant to extract essential nutrients for growth and development. Xylanases have also been found to contribute to the softening of fruits, including papaya. During the ripening process of fruits, endoxylanases, a type of xylanase enzyme, modify the polysaccharides present in the cell wall matrix. This modification leads to the softening of the fruit, making it more palatable and digestible (Manenoi & Paull, 2007).

The potential role of xylanases in fruit ripening opens up possibilities for their commercial application. It is speculated that xylanases could be used to induce ripening in fruits, enabling them to be harvested in an unripe state and then ripened artificially at their destination. This approach would allow for better fruit quality control and extended shelf life during transportation (Manenoi & Paull, 2007).

8.6 Degumming process

Pectinases and xylanases play crucial roles in various industrial processes, including the degumming and separation of bast fibers in textile production and the debarking of wood. In the context of textile production, pectinases and xylanases are used to facilitate the degumming and improved separation of bast fibers. Bamboo materials, known for their potential in textile production, benefit from enzyme hydrolysis facilitated by pectinases and xylanases (Fu et al., 2008). For instance, a study demonstrated the successful degumming of ramie bast fibers using xylanases derived from Bacillus subtilis B10 (Huang et al., 2006). These enzymes help break down the pectin and xylan components in the fiber, facilitating their separation and improving the quality of the resulting textile materials.

Furthermore, pectinases and xylanases find applications in the debarking of wood. During wood processing, debarking is an essential step that involves the removal of bark from the surface of the wood. Pectinases and xylanases are employed in this process to aid in the breakdown of pectin and xylan compounds present in the bark, facilitating its removal (Wong et al., 1992).

These enzymes play key roles in enhancing the efficiency and quality of industrial processes related to textile production and wood processing by enabling the effective degumming, separation, and debarking of fibers and wood materials.

9. CONCLUSION

There is significance of xylan and xylanase enzymes in various sectors and offers insightful information about their sources, manufacturing processes, and diverse applications. Continued research and advancements in the field of xylan and xylanase production will pave the way for further innovations, leading to improved enzyme yields, cost-effectiveness, and the exploration of novel applications in the future. In the future, improved enzyme yields, cost-effectiveness, and the investigation of novel applications will be made possible by ongoing research and advancements in the field of xylan and xylanase production.

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