Chapter 12
Fungal Xylanases: Sources, Types, and Biotechnological Applications

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Abstract Xylanase is a class of hydrolytic enzymes which cleaves the linear polysaccharide, the major constituent of hemicellulose beta-1,4-xylan into xylose. The structure of xylanase is complex, repeated linear polymers of xylopyranosyl groups at numerous carbon positions with different acidic compounds or sugars. It plays a critical physiological role in plant tissue like seed germination, plant defense system, and softening of fruits. Among microbial sources, actinomycetes, fungi, bacteria, and yeast are the principal sources of xylanases. The chief xylanase producers from fungal genera include *Aspergillus*, *Coriolus versicolor*, *Fusarium*, *Phanerochaete chrysosporium*, *Trichoderma*, and *Pichia*. The commercialization of xylanase into the industry has increased significantly due to wide number of applications. They are used in paper industries, bio-bleaching of wood pulp, bioprocessing of textiles, food additives to poultry, improvement in the nutritional properties of grain feed and silage, extraction of plant oils, starch, and coffee, etc. Solid-state fermentation is an effective method for xylanase synthesis, predominantly by fungal culture due to the advantages like high productivity at low cost as it produces xylanase by consuming cheap substrate, which serve as the carbon source as a resultant total cost of the process decreases. Advancement in recombinant DNA technology

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led to the selection of xylanase-producing microorganisms which are more likely suitable for industrial applications. The advancement in the genetic engineering can help us to amend the fungal expression system for hyper-expression of the heterologous xylanase for production as well as industrial use. Using improved technical advancement systems, development of recombinant fungal expression systems by genetic approach will help in hyper-expression of xylanases and xylanase families for their production management at the industrial level.

12.1 Introduction

Xylanase (EC 3.2.1.8, beta-xylanase, beta-1,4-xylan xylanohydrolase, xylanohydrolase, beta-D-xylanase, 1,4-beta-xylan, endo-1,4-beta-D-xylanase, beta-1,4-xylanase, endo-1,4-beta-xylanase, endo-1,4-xylanase, endo-(1->4)-beta-xylan 4-xylanohydrolase) is a class of hydrolytic enzymes which cleaves the linear polysaccharide which is the major constituent of hemicellulose beta-1,4-xylan into xylose (Talamantes et al. 2016; Vogel 2018). It plays a critical physiological role in plant tissue like seed germination, plant defense system, and softening of fruits (Saleem et al. 2008). It is second most abundant natural polysaccharide consisting mainly of D-xylose as its monomeric unit commonly present in the middle lamellae and cell wall of plant cells (Saulnier et al. 2007; Caffall and Mohnen 2009). The major chain of xylan is composed of β-xylopyranose residues which covers different groups of noncellulosic polysaccharides of small monosaccharide units such as L-arabinose, D-galactose, D-glucuronic acid, D-galacturonic acid, D-glucose, D-mannose, D-xylose, etc. (de Vries and Visser 2001; Menon et al. 2010; Segato et al. 2014). Because of the complex chemical structure and heterogeneity of plant xylan, the complete degradation requires different hydrolytic enzymes having diverse mode of action and specificities. Thus, it explains the reason for arsenal production of polymer-degrading proteins (Motta et al. 2013).

The xylanolytic enzyme system which hydrolyzes the xylan comprises different hydrolytic enzymes like α-arabinofuranosidase (α-L-arabinofuranosidase, E.C.3.2.1.55), acetylxylan esterase (E.C.3.1.1.72), α-glucuronidase (α-glucosiduronase, E.C.3.2.1.139), β-xylosidase (xylan-1,4-β-xylosidase, E.C.3.2.1.37), and endoxylanase (endo-1,4-β-xylanase, E.C.3.2.1.8) (Rahman et al. 2003; Selvarajan and Veena 2017). These diverse enzymes act in cooperation for the conversion of xylan to constituent sugar molecules (Hu et al. 2011; Su et al. 2013). Out of all xylanases, endoxylanases are considered to be of extreme importance as they are directly involved in the cleaving of glycosidic bonds and liberation of small stretches of xylooligosaccharides (Dey and Roy 2018). Reliable with their side group substitutions and structural chemistry, xylanase seems to be intertwined, covalently linked, and interspersed at many points with the superimposing sheath of lignin by hydrogen bonding (Zhang 2008; Youssefian and Rahbar 2015). Xylanases are not restricted to plants; they also can be found in majority of the species of crustaceans, snails, insects, protozoans, marine algae, etc. (Kumar et al. 2016a, b; Chakdar et al. 2016). Among microbial sources, actinomycetes, fungi, bacteria, and yeast are the principal sources of xylanases (Juturu and Wu 2012).

The characteristics of various xylanase-producing bacteria and fungi are mentioned in Table 12.1. From the past few decades, the commercialization of xylanase into the industry has increased significantly due to wide number of applications. They are used in paper industries, bio-bleaching of wood pulp, bioprocessing

Table 12.1 Characteristics of some xylanase-producing microorganisms (bacteria and fungi)

Microorganisms	Optimal pH	Optimal temperature (°C)	References
Acidobacterium capsulatum	5	65	Inagaki et al. (1998)
Acrophialophora nainiana	5	50	Ximenes et al. (1999)
Acrophialophora nainiana	7	55	Martínez-Anaya and Jiménez (1998)
Acrophialophora nainiana	7.0	55	Salles et al. (2000)
Aspergillus aculeatus	4.0, 5.0	50, 50, 70	Fujimoto et al. (1995)
Aspergillus awamori	4.0–5.5	45–55	Kormelink et al. (1993)
Aspergillus fischeri	6	60	Raj and Chandra (1996)
Aspergillus fumigatus	8.5	55	Puls et al. (1999)
Aspergillus kawachii	5.5, 4.5	60, 55, 50	Ito et al. (1992)
Aspergillus lentulus	5.3	50	Kaushik et al. (2014)
Aspergillus nidulans	6	56	Salles et al. (2000)
Aspergillus nidulans	5.5, 6.0	56, 62	Fernandez-Espinar et al. (1994)
Aspergillus nidulans KK-99 ND	8.0	55	Taneja et al. (2002)
Aspergillus niger	7.5	60	Ahmad et al. (2013)
Aspergillus oryzae	4–6	50	Szendefy et al. (2006)
Aspergillus oryzae	5	60	Fernandez-Espinar et al. (1994)
Aspergillus oryzae	6	50	Kitamoto et al. (1999)
Aspergillus sojae	5.0,5.5	50	Kimura et al. (1995)
Aspergillus sp. 26	5.0	50	Khanna et al. (1995)
Aspergillus sydowii	2–12	30	Nair et al. (2008)
Aspergillus sydowii	4	50	Ghosh and Nanda (1994)
Aspergillus terreus	4.5	45	Kimura et al. (1995)
Aspergillus terreus	6	50	Moreira et al. (2013)
Aspergillus terreus	7	50	Ghanem et al. (2000)
Aspergillus terreus	4.5	45	Ghareib and El Dein (1992)
Aspergillus versicolor	6	55	Carmona et al. (1998)
Aureobasidium pullulans	4.4	54	Li et al. (1993)
Bacillus circulans	6–7	80	Dhillon et al. (2000)
Bacillus licheniformis	7.5	50	Liu et al. (2012)
Bacillus pumilus	8.0	37	Battan et al. (2007)
Bacillus sp.	6.0	75	Bataillon et al. (2000)
Chaetomium cellulolyticum	6.5	50	Baraznenok et al. (1999)
Chaetomium cellulolyticum	5.0–7.0	50	Baraznenok et al. (1999)
Cryptococcus albidus	5	25	Morosoli et al. (1987)
Cryptococcus sp.	2.0	40	Iefuji et al. (1996)

(continued)

Table 12.1 (continued)

Microorganisms	Optimal pH	Optimal temperature (°C)	References
Fusarium oxysporum F3	6.0	60, 55	Christakopoulos et al. (1996)
Geobacillus stearothermophilus	6	60	Bibi et al. (2014)
H. grisea var. thermoidea	5.5	70	Monti et al. (1991)
Myceliophthora sp.	6	75	Chadha et al. (2004)
Paecilomyces variotii	5	60	Cesar and Mrša (1996)
Paenibacillus terrae HPL-003	4–11	55	Song et al. (2014)
Penicillium brasilianum IBT 20888	ND	ND	Jørgensen et al. (2003)
Penicillium capsulatum 22	3.8	48	Ryan et al. (2003)
Penicillium oxalicum	9	55	Dwivedi et al. (2009)
Penicillium sp.40	2.0	50	Kimura et al. (2000)
Promicromonospora sp. MARS	8	65	Kumar et al. (2011)
Schizophyllum commune	5.5	50	Kolenová et al. (2005)
Streptomyces sp.	6.0–8.0	55–60	Georis et al. (2000)
Thermoascus aurantiacus	4.0–5.0	70–75	Kalogeris et al. (1998)
Thermomyces lanuginosus	6.5	65	Ziaie-Shirkolaee et al. (2008)
Thermomyces lanuginosus	6.0–6.5	70	Singh et al. (2000)
Thermotoga maritima MSB8	6.5	55	Winterhalter and Liebl (1995)
Trichoderma harzianum	5.0	50	Tan et al. (1985)
Trichosporon cutaneum SL 409	6.5	50	Liu et al. (1998)

of textiles, food additives to poultry, improvement in the nutritional properties of grain feed and silage, extraction of plant oils, starch, and coffee, etc. (Yadav 2015; Motta et al. 2013; Goswami and Rawat 2015). Apart from these wider applications, xylanases also have potential for application in bakery processes and fruit juice processing units (Butt et al. 2008; Harris and Ramalingam 2010). The production of xylanase levels in filamentous fungi is very much higher than those found in actinomycetes, bacteria, and yeasts as they secrete xylanase directly into the medium without any processes by eliminating the need for cell disruption (Sepahy et al. 2011). Filamentous fungi also produce auxiliary enzymes which are essential for the degradation/debranching of substituted xylans (Nair and Shashidhar 2008; Brink and Vries 2011). The objective of this chapter is to discuss the various types and sources of xylanases, their industrial applications, and factors affecting the production of xylanases.

12.2 Types of Xylanases

Xylanases have been broadly classified in at least three ways: the crystal structure (Jeffries 1996), product profile or the substrate specificity and kinetic properties (Motta et al. 2013), and based on the isoelectric point and molecular weight (Wong

et al. 1988). The acceptable system for the classification of xylanases is simply based on the comparison of the catalytic domains and its primary structure. According to the CAZy database (http://www.cazy.org), xylanases (EC3.2.1.8) are linked to glycoside hydrolase (GH) families 5, 7, 8, 9, 10, 11, 12, 16, 26, 30, 43, 44, 51, and 62. Out of these, xylanases GH 10 and 11 are the two families which were extensively studied. GH family 10 comprises endo-1,3-β-xylanases and endo-1,4-β-xylanases (Motta et al. 2013). These members of the family possess the ability to hydrolyze the aryl β-glycosides at the aglyconc bond within xylobiose and xylotriose (Heo et al. 2004; Qing and Wyman 2011). On the basis of amino acid similarity index, xylanases are classified under glycoside hydrolases into families 10 and 11. It has been documented that GH10 xylanases have low pI and molecular weight ≥30 kDa, whereas GH11 xylanases have high pI and molecular weight 20 kDa approximately. Moreover, enhanced activity of these enzymes is observed on small stretches of xylooligosaccharides, thus indicating the presence of small substrate-binding site (Henrissat 1991; Gallardo et al. 2004; Murphy et al. 2011; Mathur et al. 2015). Family 11 is made up of xylanases and stated to be "true xylanases" as they are highly active on substrate having d-xylose (Liu and Kokare 2017). Among all xylanases, endoxylanases are considered to be of extreme importance as they are directly involved in hydrolyzing of glycosidic bond and liberating small stretches of xylooligosaccharides (Collins et al. 2005a). *Bacillus* species have been reported to secrete large amount of extracellular xylanase (Beg et al. 2001), along with filamentous fungi like *Aspergillus*, *Penicillium*, and *Trichoderm*a which also secretes large amount of extracellular xylanases accompanied by cellulolytic enzymes (Kohli et al. 2001; Polizeli et al. 2005; Wong and Saddler 1992).

12.3 Xylanase Structure

Xylanases are ubiquitous in nature; they are reported from rumen bacteria, terrestrial bacteria, crustaceans, snails, marine algae, insects, germinating seeds, rumen bacteria, protozoa, and fungi (Walia et al. 2015). The structure of xylanases is assumed to be 8 TIM-barrel fold of 8 parallel α strands of 32.5 kDa polypeptide chain forming cylinder-like structure followed by eight main α helices (Natesh et al. 1999). The structure of xylanase is complex, repeated linear polymers of xylopyranosyl groups at numerous carbon positions with different acidic compounds or sugars. The efficient and complete hydrolysis of the polymer needs an array of different enzymes with diverse mode of action and specificity (Segato et al. 2014). Endo-1,4-b D-xylanase (E.C. 3.2.1.8) haphazardly cleaves the xylan backbone, and xylosidases degrade the monomers of the xylose. α-L-arabinofuranosidases play an important role in the removal of the side groups, and the phenolic and acetyl side branches were removed by acetylxylan esterases, and they act on complex polymer (Drzewiecki et al. 2010; Takahashi et al. 2013). The conversion of xylan into its constituent sugar is supported by all these enzymes, and such kind of multifunctional system is commonly found in actinomycetes (Walia et al. 2015), bacteria (Azeri et al. 2010), and fungal species (Driss et al. 2011) (Fig. 12.1).

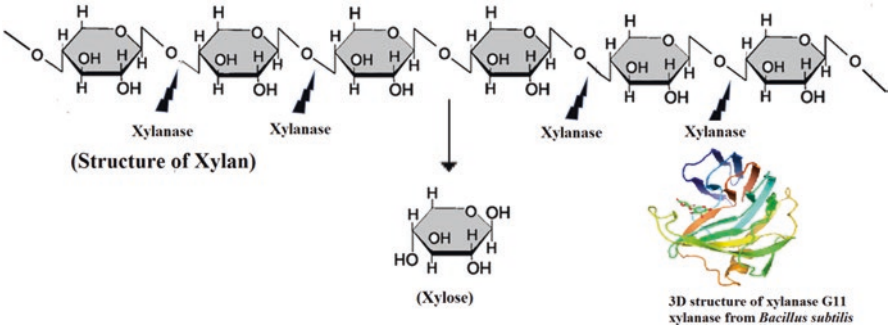

Fig. 12.1 Conversion of xylan into its constituent sugar (xylose) by xylanase enzyme (Biochem draw 12.0)

12.4 Fungal Xylanases

Advancement in research on fungus that utilizes xylan, and on its substituted enzyme systems involved, is becoming more and more relevant in economic and ecological terms. Xylanases are synthesized by both thermophiles and mesophiles (Smith et al. 1991). The chief xylanase producers from fungal genera includes *Aspergillus*, *Penicillium*, *Fusarium*, *Trichoderma*, and *Pichia* (Yadav et al. 2018; Kavya and Padmavathi 2009; Sakthiselvan et al. 2014). White-rot fungi have been reported to synthesize extracellular xylanase which can act on broad range of hemicellulose materials such as the following: *Coriolus versicolor* synthesize mixture of xylanolytic enzyme and *Phanerochaete chrysosporium* synthesize α-glucuronidase in large amount (Castanares et al. 1995; El-Nasser et al. 1997). Among the mesophilic fungi, *Trichoderma* and *Aspergillus* are the two genera which are preeminent in xylanase production (Shah and Madamwar 2005; Alvarez-Zúñiga et al. 2017). In the past few decades, lots of steps and effort have been put to isolate extremophilic and thermophilic xylanase-producing bacteria of high stability (Monti et al. 2003; Bruins et al. 2001; Rizzatti et al. 2001; Maheshwari et al. 2000; Puchart et al. 1999; Niehaus et al. 1999; Andrade et al. 1999; Kalogeris et al. 1998). Various species of thermophilic fungi have been reported which include *Thermoascus aurantiacus*, *Thermomyces lanuginosus*, *Talaromyces emersonii*, *Talaromyces byssochlamydoides*, *Paecilomyces variotii*, *Melanocarpus albomyces*, *Humicola grisea*, *Humicola lanuginosa*, *Humicola insolens*, and *Chaetomium thermophile* (Ishihara et al. 1997; Polizeli et al. 2005; Li et al. 2011; Saxena et al. 2016).

All these species of xylanase-producing fungus retain temperature between 60 °C and 80 °C and are highly stable (Amir et al. 2013). Even the enzyme produced by archaea and eubacteria is stable at high temperature, but the amount of enzyme is comparatively low in comparison to fungi (Nigam 2013). Generally, the xylanase is more in fungal culture to that of bacteria and yeast. These are mostly glycoproteins

and highly active at pH (4.5 to 6.5). They have molecular weight ranging from 6 to 38 kDa and exist in multiple forms (Chakdar et al. 2016). Although it has been also reported that the degree of structural homology is similar in endoxylanases of thermophiles and mesophiles (Collins et al. 2005b; Meruelo et al. 2012). Various authors put forth the reason behind the high stability of xylanases in thermophiles is mainly due to the presence of N-terminal proline which changes reduction in conformational freedom, extra disulfide bridges, salt bridges, and presence of hydrophobic sides (Wang et al. 2014; Panja et al. 2015). Later on, Hakulinen et al. (2003) studied that the thermal stability of xylanases is strictly based on the higher Thr/Ser ratio and the number of charged residues which results in enhance polar interactions.

From fungal kingdom, the genus *Aspergillus* is considered to be the potent producer of both β-D-xylosidase and xylanase enzyme, and moreover it has been well-characterized (Knob et al. 2010; Chakdar et al. 2016). These filamentous fungi are of industrial importance as synthesized xylanases are extracellular in nature. Additionally, fungal species have high yield in contrast to bacteria and yeast (Motta et al. 2013; Patel and Savanth 2015). On exploring xylan-degrading enzyme, many new enzymes with unique characteristics for microbes were discovered which attained the attention of industries for various applications (Nigam 2013; Anbu et al. 2017). Thermophilic fungi, unique microbes which are able to survive at high temperature, are generally associated with heaps of agricultural and forestry products. The colonization and distribution of thermostable fungal population present in the compost largely depend on a variety of degrading enzymes as fungal strains perform the enhanced function in lignocellulose waste on xylan present in it (Maheshwari et al. 2000; Singh et al. 2016a). Each enzyme has its specialized function as well as biological importance (Ali et al. 2017). Xylanases produced by thermophilic fungi which are active at alkaline pH have found their application in paper and pulp industry during bleaching process and eliminating the need of chlorine; as a result, the process is becoming eco-friendly (Raghukumar et al. 2004; Medeiros et al. 2007; Harris and Ramalingam 2010; Gangwar et al. 2014; Kumar et al. 2016a, b).

12.5 Xylanase Production

Two methods, i.e., solid-state and submerged fermentation, are most commonly used for the production of xylanases. It has been observed that production of enzyme is relatively high in solid-state fermentation (SSF) in comparison with submerged fermentation (Suman et al. 2015; Alberton et al. 2009; Ling Ho and Heng 2015). Therefore, in recent years, SSF has gained more attention by researcher because of commercial and engineering advantages (Subramaniyam and Vimala 2012). SSF can be executed on various lignocellulosic wastes like corncob, ragi bran, rice bran, soya bran, and wheat bran and have been found effective substrate for xylanase production (Kavya and Padmavathi 2009; Soccol et al. 2017). Thus, SSF is an

effective method for xylanase synthesis, predominantly by fungal culture due to the advantages like high productivity at low cost as it produces xylanase by consuming cheap substrate, which serves as the carbon source as a resultant total cost of the process decreases (Harris and Ramalingam 2010; Walia et al. 2017). Therefore, in order to reduce the cost of xylanase synthesis, lignocellulosic waste can be used as substrate instead of pure xylans (Goyal et al. 2008; Motta et al. 2013).

12.6 Application of Xylanases

From the past few decades, the biotechnological and commercial use of xylanase enzymes has increased remarkably. The major applications of xylanases are in food industries, paper industries, feed industries, biofuel production, and pharmaceutical industries (Singh et al. 2016b; Yadav et al. 2015a, b; Pedersen et al. 2015; Ahlawat et al. 2007). Xylanases are also commercially produced in developed countries such as the USA, Canada, Denmark, the Republic of Ireland, Germany, Finland, and Japan (Bajpai 2014). The commonly used microorganisms used for this purpose include *Humicola insolens*, *Aspergillus niger*, and *Trichoderma* spp. (Polizeli et al. 2005; Harris and Ramalingam 2010). In the future, it might be used for the biodegradation of organic (Shukla et al. 2016; Kumar et al. 2017; Singh et al. 2017a, b, Kaur et al. 2017) and inorganic contaminants (Kumar et al. 2015a; Mishra et al. 2016; Singh et al. 2016b; Kumar et al. 2016a, Kumar et al. 2016b) such as pesticides (Kumar et al. 2013, 2014b) heavy metal, etc. (Kumar et al. 2014c. Kumar et al. 2015b). However, no study is reported till date. Before 1980, it was used in the preparation of the feeds for animals. Nowadays, xylanase along with cellulose and pectinase accounts for more than 20% of enzyme market worldwide (Choct 2006; M'hamdi et al. 2014; Sahay et al. 2017). Presently, some industries have put forth their interest in the development of various efficient enzymatic processes which could replace acid hydrolysis treatment of hemicellulose-containing material (Hu et al. 2011). The major application of xylanases in industries and their uses were described in Table 12.2.

Due to biotechnological potential of xylanase, it has aroused the great interest in the industrial sector like ethanol and xylitol synthesis in paper and cellulose industry and liquid fuel, cellular protein, and chemical production in food industry (Yadav et al. 2017a, b, c, d; Kulkarni et al. 1999; Guimaraes et al. 2013). Most of the agricultural waste comprises of cellulose and hemicellulose which needs to be converted in constituent sugar (Anwar et al. 2014; Saini et al. 2015). Waste synthesized by agro-industry and food industry is available in staggered amount all over the world and is becoming the health hazard (Kanimozhi and Nagalakshmi 2014). In order to utilize the waste, we require strategic planning and chemicals for hydrolyzing the constituent (Paritosh et al. 2017). Due to xylan being the major polymer in the plant structure, xylanases and microbes producing xylanase enzyme can be adapted for processing of food, paper pulp, sugar, ethanol, and agro-industries (Sridevi et al. 2016; Walia et al. 2017).

Table 12.2 Commercial production of different xylanases with their trade name and industrial applications

Trademark/name	Company/supplier name	Application and uses	Country of origin
Allzyme PT	Alltech	Feed industry	America
Amano 90	Amano	Pharmaceutical industry	Japan
Biofeed	Novo Nordisk	Feed industry	Denmark
Biofeed Plus	Novo Nordisk	Feed industry	Denmark
Bleachzyme F	Bicon	Paper industry	India
Cartazyme	Sandoz Charlotte, N.C.	Paper industry	Switzerland
Ceremix	Novo Nordisk	Food industry	Denmark
Ecopulp	AlkoRajamaki	Paper industry	Finland
Ecopulp	Rohn Enzyme 0Y, Primalco	Paper industry	Finland
Ecosane	Biotec	Feed industry	Thailand
Ecozyme	Thomas Swan	Paper industry	UK
Enzekoxylanase	Enzyme Development	Feed industry	USA
Gamazyme X4000L	Gamma Chemie GmbH	Brewing industry	Germany
Grindazym GP 5000	Danisco Ingredients	Feed industry	Denmark
Grindazym GP e GV	Danisco Ingredients	Feed industry	India
GS-35, HS70	Iogen	Paper industry	Canada
Irgazyme 40	Nalco-Genencor, Ciba	Paper industry	Geigy
Multifect XL	Genencor	Food industry	Netherlands
Pulpzyme, Sanzyme PX	Novozymes	Paper industry	Denmark
Alpelase F	Sankyo	Paper industry	Japan
Sanzyme X	Sankyo	Food industry	Japan
Sternzym HC 46	Stern-Enzym	Feed industry	Mexico
Optipulp L-8000	Solvay Interox	Food industry	USA
Rholase 7118	Rohm	Food industry	Germany
Solvay pentonase	Solvay Enzymes	Food industry	Canada
VAI Xylanase	Voest Alpine	Paper industry	Austria
Xylanase	Meito Sankyo	Research	Nagoya, Japan
Xylanase250	Hankyo Bioindustry Co. Ltd	Baking industry	Japan

For the production of ethanol, first delignification of the lignocellulose biomass is required, followed by the hydrolysis of cellulose and hemicellulose polymer to monosaccharide sugar (Lee et al. 2014; Kumar and Sharma 2017). Hydrolysis can be conducted either by acid treatment at elevated temperature or action of enzyme. If the acid hydrolysis procedure is assessed in context to cost, it becomes expensive because of energy consumption and equipment (Woiciechowski et al. 2002; Timung et al. 2016; Amin et al. 2017). The lignocellulosic biomass comprises complex constituent that requires action of various enzymes like β-glucosidases,

β-xylosidases, endoglucanases, and xylanases in synergistic manner for proper hydrolysis (Yeoman et al. 2010; Hu et al. 2011). Xylanase also has the application in paper and pulp industry for bleaching of kraft pulp (Azeri et al. 2010). Generally, xylanase documented till date is found to be effective at neutral pH 6 and temperature 50 °C (Chakdar et al. 2016). In enzyme associated with pulp bleaching process, the temperature and pH of incoming pulp are high, thus making the thermostable alkaline xylanase the enzyme of interest (Kumar et al. 2014a; Cunha et al. 2018a). Moreover, usage of xylanase in paper industry during bleaching processes decreased the usage of chemicals and gives enhanced brightness to paper (Sharma et al. 2017).

For various processes like juice clarification, extraction of coffee, plant oils, and starch requires the amalgam of pectinase, xylanase, and other enzymes (Goswami and Rawat 2015; Tallapragada and Venkatesh 2017). Xylanases have various potentials in various industries like paper, animal, food, and biofuel industries (Beg et al. 2001; Polizeli et al. 2005; Harris and Ramalingam 2010). During the formulation of feed, xylanase along with amylase, glucanase, and pectinase decreases the feed viscosity and elevates the nutrient adsorption. Generally, the nutrients are liberated by hydrolyzing the nondegradable fibers by enzyme, or they liberate the enzyme arrested by fibers (Mathlouthi et al. 2002).

In the last few decades, xylanolytic enzymes have also attained their importance in bread-making industry (Butt et al. 2008), in which non-starch and starch hydrolyzing enzyme is predominantly used for improving the bread quality. Xylanases have been reported to enhance tolerance of dough to diverse flour quality parameters as well as the amendment in processing methods (Ahmad et al. 2014; Cunha et al. 2018b). They make the dough softer, decrease the work supplies, and increase the quantity of leavened pan bread (Jaekel et al. 2012). These xylanolytic complexes have their role in textile industries for plant fiber processing in case of linen and hessian (Polizeli et al. 2005). Thus, the overall scenario favors and depicts that fungal xylanases have great potential and industrial advantages and in association with other enzymes can aid in gaining profit for industries (Walia et al. 2017; Kumar et al. 2018).

12.7 Cloning of Fungal Xylanase Genes

Advancement in recombinant DNA technology led to the selection of xylanase-producing bacteria which are more likely suitable for industrial applications (Singh et al. 2016b). The key challenge for this technology includes the production of xylanolytic systems and upgrading of fermentation characteristic of bacterial and fungal species by inserting genes for xylosidase and xylanase (Knob et al. 2014; Kapilan and Arasaratnam 2017). Filamentous fungi come in the category of xylanase producers which show both homologous and heterologous gene expression.

Their promoter region expresses the enzymes with high yields. It's not possible to attain particular enzyme in its pure form (Ahmed et al. 2009; Mustafa et al. 2016). Therefore, such technology can be applied to achieve such purposes. The genes coded for xylanases have been cloned in heterologous and homologous hosts with the intention to overproduce the enzyme and change its property to be best suited for industrial applications (Lambertz et al. 2014; Walia et al. 2017). Various genes have been cloned and expressed to enhance the production of enzymes, their specificity, substrate utilization, and other industrial applications. *E. coli* has been selected worldwide for heterologous or homologous expression of recombinant proteins and gene cloning in xylanase-producing organisms (Adrio and Demain 2014; Chakdar et al. 2016). This is due to its widespread cloning vectors, ease of DNA cloning, secretion of homologous proteins, and overproduction of recombinant proteins directly into the natural hosts. They are used since long times for production of recombinant enzymes either extracellularly or intracellularly (Walia et al. 2017). The major drawback of using *E.coli* as expression vector is that some of the proteins are not secreted efficiently (Rosano and Ceccarelli 2014).

However, *E.coli* has been found as virtuous host for recombinant protein for cloning xylanase genes and can be further used to carry out its gene structure (Reeves et al. 2000). Other microbes such as *S. cerevisiae* and *P. pastoris* are also used to secrete high amount of xylanase production in batch mode medium at low cost (Damaso et al. 2003; Shang et al. 2017). Due to high-expression characteristics, they both emerge as excellent host under its own promoters. One of the major drawbacks of both the species is its use in large-scale production and health hazards of methanol (Motta et al. 2013; Walia et al. 2017).

Usage of xylanases for various roles largely depends on the kinetics, pH stability, and optimum temperature (Liao et al. 2015). The recombinant xylanases synthesized by fungal and yeast strains have been reported to show equivalent or enhanced properties than the native enzymes. Thermostable enzymes are employed in the various processes in the industry, but propagation of thermostable microbes is found to be ineffective at large scale because of extreme fermentation conditions (Damaso et al. 2003; Kumar et al. 2016a, b). It has been reported that *T. reesei* and *P. pastoris* express the thermostable xylanase at a high level (Mellitzer et al. 2012; Huang et al. 2012). In the same way, anaerobic microbes also show the expression of xylanase and thus can be used in the fermentation industry. There are chances for unraveling the new strains of fungi which can produce recombinant xylanases (Motta et al. 2013; Nigam 2013).

Moreover, the advancement in the genetic engineering can help us to amend the fungal expression system for hyper-expression of the heterologous xylanase for production as well as industrial use. Sometimes, overexpression of recombinant proteins led to site-direct mutagenesis using recombinant technology (Kim et al. 2012; Lambertz et al. 2014). Lists of various fungal species along with their cloning vectors and hosts are depicted in Table 12.3.

Table 12.3 Recombinant DNA technology in gene cloning of different fungal xylanase genes in fungi

Source organism	Gene	Vector	Host	Molecular mass of the recombinant enzyme (kDa)	Characteristics of the recombinant enzyme (pH, temperature, K_m, V_{max}) [secretion level, enzyme activity] (stability)	References
Acrophialophora nainiana	xyn6	pHEN11 exp. (pUC19-based)	*Trichoderma reesei* Rut C-30	19	172 mg L^{-1} (secretion level)	Salles et al. (2007)
Aspergillus awamori ATCC11358	exlA	pAW14S	*Aspergillus awamori*	–	–	Hessing et al. (1994)
Aspergillus niger biAI	xlnD	pUC18 (pXDEI) pGW635	*Aspergillus nidulans* G191	–	–	Pérez-González et al. (1998)
Aspergillus niger BRFM281	xynB	pAN52.3	*Aspergillus niger* D15#26	23	(5.5, 50, 7.1 mg mL^{-1}, 3881 U mg^{-1}), [900 mg L^{-1}]	Levasseur et al. (2005)
Aspergillus oryzae KBN 616	xynG2	pNAN814	*Aspergillus oryzae* KBN616-ND1	21	(6.0, 58 °C, 7.1 mg mL^{-1}, 123 μmol min^{-1} mg^{-1})	Kimura et al. (1998)
Aspergillus oryzae KBN 616	xynF3	pNAN8142	*Aspergillus oryzae* KBN616-ND1	32	(5.0, 58 °C, 6.5 mg mL^{-1}, 435 μmol min^{-1} mL^{-1})	Kimura et al. (2002)
Aspergillus oryzae KBN 616	xynF1	pXPR64 (pUC118 based)	*Aspergillus oryzae* KBN616-39	ND	ND	Kitamoto et al. (1998)
Aspergillus oryzae KBN 616	xynF1	pTFXF200 (pUC19 based)	*Aspergillus oryzae* KBN616-39	35	(5.0, 60) [180 mg L^{-1}]	Kitamoto et al. (1999)
Aspergillus oryzae KBN 616	xynGI	pDJB1	*Aspergillus nidulans* G191	–	–	Kimura et al. (1998)

Chaetomium gracile IFO6568	cgxA cgxB	pDJB1	*Aspergillus nidulans* G191	–	–	Yoshino et al. (1995)
Chaetomium thermophilum CBS730.95	Ctxyn11A Ctxyn11B Ctxyn11C	pUC19	*Trichoderma reesei* ALK04468	27 23 22	(6, 70 °C) [148 ukat/mL, 9.2 mg mL^{-1}], {>90% at 80 °C, pH 5–6}; (6, 70 °C) [57.7μkat mL^{-1}], {<40% at 80 °C}, [1.4 7 μkat mL^{-1}]	Mäntylä et al. (2007)
Cochliobolus carbonum	xyl2 xyl3	pXLB37-2 pHYG2	*Cochliobolus carbonum* XYL mutant strain	–	–	Apel-Birkhold and Walton (1996)
Cochliobolus carbonum	xyl1	pCC167	*Cochliobolus carbonum* XYL mutant strain	–	20.8	Apel et al. (1993)
Humicola grisea var. thermoidea	xyn2	pHEN	*Trichoderma reesei* HEP1	–	(6.5, 70 °C) [500 mg L^{-1}, 12,700 nkat mL^{-1}]	de Faria et al. (2002)
Orpinomyces sp. PC-2	xynA	pT3C	*Trichoderma reesei* Rut C-30	–	28,150 mg L^{-1} (secretion level) 1250–1700 s^{-1} (kcat)	Li et al. (2007)
Phanerochaete chrysosporium RP78	xynA xynB xynC	ANEp2	*Aspergillus niger* N593	52 32 50	(4.5, 70, 3.42 mg mL^{-1}), (4.5, 60, 9.96 mg mL^{-1}) (4.5, 70, 3.71)	Decelle et al. (2004)
Trichoderma reesei ALK02721 ALK02221 VTT-D-79125	xln2	pBluescript, pUC19	*Trichoderma reesei*		[3700 nkat mL^{-1}] [3800 nkat mL^{-1}] [10,000 nkat mL^{-1}]	Saarelainen et al. (1993)

12.8 Conclusions and Future Prospects

Xylanases have extensive range of application in various industries such as paper, pulp, animal feed, pharmaceutical, and pulp industries. Due to its varying properties of hydrolysis and low toxicity, they are also used in food industry. It also reduces load of chemical additives and emulsifiers in food industry. The current review shows that production of xylanases in large-scale production is still a challenging task. New approaches, such as consensus polymerase chain reaction screening of genome sequencing, functional approaches, and study of extremophilic enzymes, will further add new prospects to understand the other applications of the xylanase. There is also possibility of isolating new fungal species for producing recombinant xylanases. Using improved technical advancement systems, development of recombinant fungal expression systems by genetic approach will help in hyper-expression of xylanases and xylanase families for their production management at the industrial level.

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