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A SHORT REVIEW ON ACETYL XYLAN ESTERASES

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Abstract

Lignocellulose is a resource for renewable organic matter. Between the three main components, hemicellulose is the second most abundant natural polymer on earth, its main constituent being xylan. Acetyl xylan esterases are accessory enzymes involved in biodegradation of xylan, releasing acetic acid from side chains of xylan backbone. After the action of these enzymes, other lignocellulases are able to act on their specific substrate. The main microbial sources for acetyl xylan esterase include Penicillium sp., Thermoanarobacterium sp., Aspergillus sp., Fusarium sp., Streptomyces sp., Phanerochaete sp., Bacillus sp., Trichoderma sp. etc. Screening methods for identification of acetyl xylan esterase microorganisms requires specific substrate for this enzyme such as acetylated xylan or xylooligosaccharides, α and β-naphthyl acetate or p-nitrophenyl acetate. The importance of these enzymes is given by their role in various applications such as biofuel production, pulp and paper biobleaching or food and feed.

Key words*: acetyl, esterase, lignocellulose, xylan, xylanase.*

INTRODUCTION

Plant biomass, mostly represented by lignocellulose, is one of the most abundant biomasses on Earth. Lignocellulose degradation is still a top research subject, due to its potential for biofuel, biodegradable plastics, organic acids (Dumitru et al., 2018; Trulea et al., 2016) or other value-added compounds.

Lignocellulose is comprised mostly of cellulose, hemicellulose and lignin. Between the three main components of lignocellulose, hemicellulose is the second most abundant polymer.

Hemicellulases are enzymes that catalyse hemicelluloses degradation acting either as glycoside hydrolases or carbohydrate esterases (e.g. acetyl xylan esterases) (Chiș et al., 2010).

According to several reports, the potential worldwide market value for hemicellulose was estimated to almost 178 million € (Wysokińska, 2010), with the condition that hemicellulose is depolymerised to pure forms of oligosaccharides or monosaccharide (Sista Kameshwar & Qin, 2018).

Xylan forms hemicellulose and is mainly found in plant cell wall (Ciotea & Popa, 2019). Its depolymerisation requires the combined action of a group of enzymes generally known as xylanases. Among them, acetyl xylan esterase is an accessory enzyme important for deacetylation of xylo-oligosaccharides and xylans.

Acetyl groups increase the plant resistance to the action of lignocellulosic enzymes (Biely et al., 2013). Therefore, acetylation has a crucial role in establishing the physio-chemical properties of the cell wall, such as: water solubility, recalcitrance to degradation and bulk volume of polysaccharide.

Removing the acetyl groups from xylan structure will lead to exposed areas susceptible to hydrolysation by other enzymes such as xylanases and in the end will increase cellulases accessibility (Sista Kameshwar & Qin, 2018).

Although there are several pretreatment methods that can remove acetyl groups from lignocellulosic structures, most of these methods have some disadvantages such as: economic viability (Adesioye et al., 2016), environmental impact or harsh experimental conditions (Diguta et al., 2007). Therefore, there's a necessity for developing a method that can overcome these obstacles, one possibility being the enzymatic hydrolysis of these acetyl groups with acetyl xylan esterases.

CHARACTERISTICS OF ACETYL XYLAN ESTERASES

Acetyl xylan esterase (E.C. 3.1.1.72, AcXE, AXE) catalyses the hydrolysis of acetyl side-chain groups linked to xylan backbone, as seen in Figure 1.

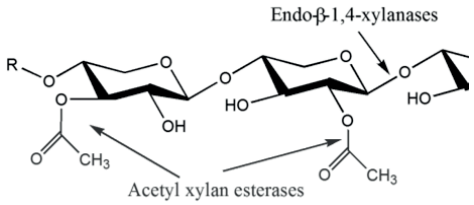

Figure 1. Hydrolysis of xylan by endoxylanases and acetyl xylan esterases (Wu et al., 2017)

The CAZy database integrate acetyl xylan esterases in carbohydrate esterase families CE 1-7 and 16-17. Most of these enzymes need for deacetylation a catalytic triad of Ser-His-Asp, with the exception of CE 4 family that entails a different mechanism, by using a metal-ion dependent hydrolysis (Mai-Gisondi & Master, 2017).

Acetyl xylan esterases were first recognized as part of xylanolytic and cellulolytic systems since 1985 by Biely, described as enzymes able to remove acetyl groups from D-xylopyranosyl residues (Biely & Côté, 2005).

Several studies (Biely & Côté, 2005; Sista Kameshwar & Qin, 2018; Zhang et al., 2011) suggest that the complete degradation of xylan by endoxylanases proceeded faster and with a higher level after deacetylation with acetyl xylan esterases. Also, the synergistic action of cellulase, endoxylanase and AXE resulted in an improved hydrolysis of cellulose, highlighting the intricate structure of acetylated xylan interlinked with cellulose fibrils (Sista Kameshwar & Qin, 2018).

The usual substrates subjected to the action of acetyl xylan esterase are: O-acetyl-4-O-methyl-D-glucurono-D-xylan (acetyl xylan found hardwood hemicellulose), acetylated xylan (Johnson et al., 1988), acetylated glucose, acetylated xylose, alpha-napthyl acetate or p-nitrophenyl acetate. Substrate specificity of acetyl xylan esterases is not well understood yet, due to lack of knowledge regarding the relationship between structure and function (Biely & Côté, 2005).

SCREENING FOR ACETYL XYLAN ESTERASE ACTIVITY

There are different screening methods used to identify the microbial producers that exhibit acetyl xylan esterase activity, most of them using fluorogenic or chromogenic acetyl esterase substrates.

The qualitative screenings of AXE are plate screening methods that are based on cultivating the microbial strain on a minimal agar medium with an unique carbon source such as: acetylated xylan, p-nitrophenyl acetate, α- or β-naphthyl acetate or 4-methylumbelliferyl acetate (Biely & Côté, 2005; Martínez-Martínez et al., 2007). After incubation, AXE activity can be identified as a hydrolysis zone around the microbial colony.

For a quantitative assay of AXE activity, the substrate can be natural or synthesized: p-nitrophenyl acetate (Atta et al., 2011), α-naphthyl acetate, N, N'-diacetylchitobiose, acetylated xylan, cellulose pentaacetate, galactose pentaacetate (Degrassi et al., 2000), 7-amino cephalosporanic acid (Martínez-Martínez et al., 2007).

An easy and highly reproducible assay for AXE activity is based on measuring the hydrolysis of p-nitrophenyl acetate to p-nitrophenol, as suggested by several studies (Atta et al., 2011; Burlacu et al., 2018). The assay mixture containing 1 mL 100 mM sodium phosphate buffer (pH 7.00), 0.9 mL 10 mM p-nitrophenyl acetate and 0.1 mL enzyme sample was incubated at 37ºC and after 10 minutes, the release of p-nitrophenol was measured by reading the absorbance at 410 nm. One unit of acetyl xylan esterase activity was defined as the amount of enzyme that will release one μmol of p-nitrophenol per minute under the specified assay conditions (Atta et al., 2011; Burlacu et al., 2018).

SOURCES OF ACETYL XYLAN ESTERASES

The acetylated glycosyl residues found in lignocellulosic structures protect cellulose and hemicellulose from the action of glycoside hydrolases. Thus, microorganisms were required to secrete several enzymes capable of releasing acetyl groups from these structures

known as carbohydrate esterases (CE), one of them being acetyl xylan esterases.

AXE producing microorganisms have been isolated and characterised from various environments (Adesioye et al., 2016). Microbial production of AXE was preferred to plant or animal sources due to easier genetic modifications or manipulation, availability and structural stability (Atta et al., 2011).

The bacterial strains that are known to exhibit AXE activity are included in Table 2, the main producers belonging to *Bacillus, Fibrobacter, Streptomyces* and *Thermobifida*.

Table 2. Bacterial sources of AXE

Microorganism	Literature
Acidothermus cellulolyticus	Shahid et al. (2018)
Anoxybacillus flavithermus	Eminoğlu et al. (2015)
Bacillus pumilus	Degrassi et al. (2000) Martínez-Martínez et al. (2007)
Bacillus subtilis	Tian et al. (2014), Christov & Prior (1993)
Butyrivibrio proteoclasticus	Till et al. (2013)
Caldanaerobacter subterraneus	Moriyoshi et al. (2013)
Caldicellulosiruptor saccharolyticus	Lüthi et al. (1990)
Chrysosporium lucknowense	Pouvreau et al. (2011)
Clostridium cellulovorans	Kosugi et al. (2002)
Fibrobacter succinogenes	Yoshida et al. (2010)
Flavobacterium johnsoniae	Razeq et al. (2018)
Geobacillus stearothermophilus	Lansky et al. (2014)
Hungateiclostridium thermocellum	Neumüller et al. (2015)
Ruminiclostridium josui	Wang et al. (2018)
Streptomyces sp.	Coman et al. (2013)
Streptomyces flavogriseus	Christov & Prior (1993)
Streptomyces lividans	Biely et al. (2013)
Streptomyces olivochromogenes	Christov & Prior (1993)
Thermoanaerobacterium saccharolyticum	Lorenz & Wiegel (1997)
Thermobifida fusca	Huang et al. (2010) Christov & Prior (1993)
Thermotoga maritima	Drzewiecki et al. (2010)

The most studied xylan degrading fungi (Table 3) were filamentous fungi (*Aspergillus spp., Trichoderma spp.)*, known for their ability to produce a wide range of xylanases.

Table 3. Fungal sources of AXE

Microorganism	Literature
Aspergillus awamori	Christov & Prior (1993) Koseki et al. (2005)
Aspergillus ficuum	Park (2011)
Aspergillus japonicus	Christov & Prior (1993)
Aspergillus luchuensis	Komiya et al. (2017)
Aspergillus nidulans	Mai-Gisondi et al. (2017) Christov & Prior (1993)
Aspergillus niger	Neumüller et al. (2015)
Aspergillus oryzae	Manavalan (2017)
Chrysosporium lucknowense	Pouvreau et al. (2011)
Coprinopsis cinerea	Juturu et al. (2013)
Fusarium oxysporum	Christov & Prior (1993)
Neocallimastix frontalis	Kwon et al. (2016)
Orpinomyces sp.	Comlekcioglu et al. (2014) Neumüller et al. (2015)
Penicillium chrysogenum	Yang et al. (2017)
Phanerochaete chrysosporium	Huy et al. (2013)
Rasamsonia emersonii	Neumüller et al. (2015)
Rhodotorula mucilaginosa	Christov & Prior (1993)
Schizophyllum commune	Biely et al. (2013) Christov & Prior (1993)
Talaromyces purpureogenus	Colombres et al. (2008)
Termitomyces clypeatus	Mukhopadhyay et al. (2003)
Thermothelomyces thermophilus	Kool et al. (2014)
Trichoderma longibrachiatum	Neumüller et al. (2015)
Trichoderma reesei	Biely et al. (2013) Christov & Prior (1993) Neumüller et al. (2015)
Volvariella volvacea	Liu & Ding (2016) Tian et al. (2012)

As observed, there are numerous microorganisms that display acetyl xylan esterase activity from both bacteria and fungi, strains that will secrete various hydrolases for the complete breakdown of cellulose and xylan (Sista Kameshwar & Qin, 2018).

Acetyl xylan esterase production is linked to the type of microbial strain, cultivation media composition and the fermentation protocol. Solid state fermentation (SSF) has an immense potential for AXE synthesis due to its advantages such as: higher productivity, wide variety of matrices, higher concentration and stability of the desired product, low energy consumption, easier control of contamination or less expensive process (Atta et al., 2011).

APPLICATIONS OF ACETYL XYLAN ESTERASES

An important role of acetyl xylan esterases is its synergistic action with xylanases and cellulases in lignocellulose degradation for biofuel (bioethanol) production (Sista Kameshwar & Qin, 2018).

Another application of these enzymes is linked to pulp and paper industry, were their action combined with endoxylanases activity leads to an improved biobleaching process (Sista Kameshwar & Qin, 2018), a protocol that requires less highly toxic chemical pretreatments.

By removing some of the side chains of xylan structure, including acetyl groups, the modified xylan obtained can be directed to form a hydrogel suitable for pharmaceutical use as a drug delivery agent (Van Zyl et al., 2013). Furthermore, some studies suggest that AXE can be used in deacetylation of cephalosporin C and thus in antibiotic production (Benini et al., 2001), such as cephalosporins, penicillins, monobactams and carbapenems (Sista Kameshwar & Qin, 2018).

AXE action on the highly viscous lignocellulose can lead to deacetylated xylo-oligosaccharides that are used as feed additives that will increase digestibility (Ștef et al., 2013). Also, AXE can be use as prebiotics in both food or feed industries (Motta et al., 2013). In addition, the supplementation of cellulases and xylanases, including AXE, to animal feedstock increased milk production of buffaloes and goats (Sista Kameshwar & Qin, 2018).

AXE can be used in food processing applications for clarifying fruit juices along with pectinases (Atta et al., 2011).

CONCLUSIONS

Despite its potential, lignocellulose remains relatively underutilized due to its structural complexity and recalcitrance, demanding a combined action of several various enzymes with specific mechanisms for complete degradation.

Acetyl xylan esterase are responsible for removing acetyl side-chain groups linked to xylan backbone. Deacetylation of xylan can improve cellulase access to cellulose and thus improve depolymerisation of lignocellulose and generate value-added products.

Due to scarcity of microbial producers of AXE, there's a high interest in finding new sources of acetyl xylan esterases by employing different screening protocols.

Although, AXE is considered to be an accessory enzyme, its importance is depicted from its role in different industrial applications such as food, feed, medical, biofuel or pulp and paper.

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