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BIOTECHNOLOGICAL ASPECTS OF FERULOYL ESTERASE

Aglaia POPA (BURLACU), Florentina ISRAEL-ROMING, Călina Petruța CORNEA, Maria Mihaela ZUGRAVU (MICUȚI)*

University of Agronomic Sciences and Veterinary Medicine of Bucharest,
Faculty of Biotechnology, 59 Mărăști Blvd, District 1, Bucharest, Romania

*Corresponding author email: mihaela.micuti@yahoo.com

Abstract

Industrial processes lead to high amounts of lignocellulosic wastes, that are not valorised properly. Feruloyl esterase are a group of accesory enzymes used in the biodegradation of lignocellulose, capable of releasing ferulic acid that is located either between lignin and hemicellulose or between hemicelluloses. The importance of feruloyl esterase is given mainly by the fact that its action allows other enzymes to hydrolyse xylan or lignin. The released ferulic acid has several applicatios in various industries such as food, feed, medical, biofuel, pulp and paper etc. Feruloyl esterases are produced by several microorganisms (bacteria or fungi), but research studies are still focused on finding more sources for these enzymes. There are different methods for detecting microbial strains able to produce feruloyl esterase, the most used being plate screening methods.

Key words*: ferulic acid, feruloyl esterase, lignocellulose, xylan.*

INTRODUCTION

Massive agro-industrial and food processing lead to an accumulation of lignocellulosic biomass, one of the most abundant biomasses, with almost 200 billion tons (Gunjal et al., 2020). Lignocellulosic wastes, are either discarded or used as animal feed (Sarangi & Sahoo, 2010), without being properly valorised (Gropoșilă-Constantinescu et al., 2017; Trulea et al., 2016). The microbial degradation of these lignocellulosic structures is considered to be the best alternative to obtain valuable compounds, without affecting the environment and using low-cost processes. One major impediment in lignocellulose valorization is linked to its recalcitrance and structural complexity, making it difficult to depolymerize.

The structural complexity of lignocellulose is given mainly by its components: cellulose, hemicellulose and lignin (Dumitru et al., 2018). Therefore, for a complete depolymerisation several main enzymatic systems are required: cellulases, xylanases and ligninases.

Xylanases are a group of enzymes with the ability to completely hydrolyse xylan (hemicellulose's main component) into smaller fragments, being divided into two categories: primary enzymes (endo β-1,4 xylanase and β-xylosidase) and accessory enzymes (α-L-arabinofuranosidase, α-glucuronidase, acetyl xylan esterase, ferulic esterase and p-coumaric acid esterase (Xue et al., 2012). Although most of them are labelled as secondary enzymes, their activity is very important due to their capacity to remove side chains from the main xylan structure and improve the accessibility of other enzymes such as cellulases, xylanases or pectinases (de Oliveira et al., 2014).

Feruloyl esterases or ferulic acid esterases (FAE, E.C. 3.1.1.73) are a group of enzymes involved in ester bonds hydrolyzation and release of ferulic acid (4-hydroxy-3-methoxy-cinnamic acid) from lignocellulosic structures (Figure 1).

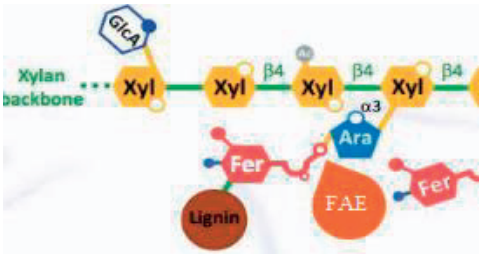

Figure 1. Feruloyl esterase mode of action (Dilokpimol et al., 2017)

Ferulic acid is either linked to arabinose or galactose residues (Figure 1) and is considered to be the most abundant and ubiquitous

hydroxycinnamic acid found in plants (Sarangi and Sahoo, 2010). As seen in Figure 1, ferulic acid is located either between hemicellulose and lignin, either between hemicelluloses (Wong, 2006). Therefore, its release could also beneficially enhance the activity of several lignin modifying enzymes (phenol oxidases or hem containing peroxidases) (Kumar and Chandra, 2020). During lignin biodegradation, FAE acts as a mediator for laccase and can also help in depolymerising polysaccharide complexes (Ozer et al., 2020).

There are many sources known as having high ferulic acid content such as: wheat, rice, oat, grains, fruits (pineapple, banana), coffee, vegetables (beetroot, spinach, artichoke), bamboo, beans, nuts etc. (Kumar & Pruthi, 2014; Topakas et al., 2007). The amount of ferulic acid from these sources varies between 0.5-2% (Kumar & Pruthi, 2014) and most of the times ferulic acid is extracted in trans isomeric form (Figure 2).

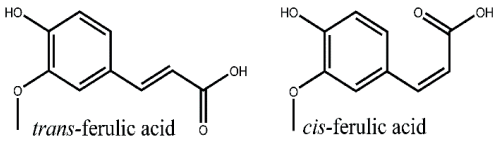

Figure 2. Ferulic acid isomeric forms (Horbury et al., 2016)

Amongst these substrates, agricultural by products are considered to be most desirable, due to economic and environmental reasons.

FERULOYL ESTERASE IMPORTANCE

The importance of feruloyl esterase derives mainly from its participation in releasing ferulic acid from lignocellulose, especially in combination with endoxylanases (Nagar et al., 2010).

Ferulic acid is used in various industrial applications such as: medical, pharmaceutical, cosmetics, food, feed, environmental etc. (Huang et al., 2011; Chen et al., 2020; Liu et al., 2020; Dilokpimol et al., 2017; Topakas et al., 2007). Its biotechnological value is related to some of its properties, such as: antimicrobial, antiallergic, anti-diabetic, antioxidant, anti-ageing, anti-inflammatory, antitumoral, neuroprotective, antithrombosis, antibiotic or even emulsifying (Sarangi & Sahoo, 2010; Liu et al., 2020; Pellerito et al., 2020; Shi et al., 2016; Sakai et al., 1999; Hong et al., 2016; Eom et al., 2016; Kumar & Pruthi, 2014; Grigore et al., 2019; Nichita et al., 2016).

Although initially ferulic acid was produced via chemical synthesis, in the last years it was considered necessary a more environmental approach via microbial fermentation.

Thus, scientists are still focused on isolating new and improved strains that display FAE activity.

Feruloyl esterases are capable of hydrolysing phenolic compounds esterified from lignocellulosic structures in a less aggressive manner, as opposed to chemical treatments that affect the environment (Jiao et al., 2014).

Other biotechnological applications of feruloyl esterase are: animal feed additives (improve nutrient assimilation), pulp and paper industry (enhance endoxylanases activity in bio-bleaching processes), bio-polymers, food industry (juice clarification, bread quality improvement, flavours etc.), bio-fuel, pharmacology etc. (Fazary & Ju, 2007; Bhathena et al., 2008; Topakas et al., 2007; Ozer et al., 2020; Sharma et al., 2020).

SCREENING PROTOCOLS OF FERULOYL ESTERASE

There are several ways to identify feruloyl esterase activity, one of the most used method being plate screening protocols.

A simple, low-cost and efficient method is described by Donaghy et al. (1998), where the microbial strains were cultivated in a minimal agar medium with the following composition (g/l): 1.3 $(NH_4)_2SO_4$, 0.25 $MgSO_4$ x 7 H_2O, 0.37 KH_2PO_4, 0.07 $CaCl_2$ x 2 H_2O, 0.03 $FeCl_3$, 1.0 yeast extract and 20 agar (for microbiological purposes). The screening media was supplemented with 0.3 ml ethyl ferulate solution (prepared in dimethylformamide), after the sterilization and partial cooling of the medium. Ethyl ferulate acted as the only carbon source, so only microorganisms that will produce FAE will be able to grow and hydrolyse the medium. After incubation at 30°C for 24 hours, the plates were analyzed based upon their hydrolysis zone around the colony, which indicated feruloyl esterase activity, as seen in Figure 3.

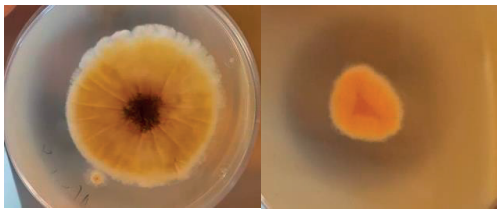

Figure 3. Hydrolysis zones around microbial colonies indicating FAE activity (original)

Although the most used and precise methods for assaying FAE activity are based on HPLC techniques (Fazary and Ju, 2007), a simple and economic protocol was described in several studies (Qi et al., 2011; Dilokpimol et al., 2017; Fazary & Ju, 2007; Mastihuba et al., 2002).
The method is based on quantifying the released ferulic acid from the hydrolysed substrate. The assay mixture was comprised of: 0.8 ml phosphate buffer 100 mM (pH = 6.00), 0.2 ml sample (from the microbial culture) and 15 µl ethyl ferulate solution (prepared in dimethylformamide).
After the incubation at 37°C for 2 h, the samples were placed in a water bath at 99°C for 3 minutes and subjected to spectrophotometric analysis at λ = 338 nm. With a ferulic acid solution (prepared in dimethylformamide) was constructed a calibration curve, that allowed to determine FAE activity. One unit of FAE was defined as the amount of enzyme that released 1 µmol of ferulic acid per minute under the assay conditions.

SOURCES OF FERULIC ACID ESTERASE

Several studies demonstrated that both fungi and bacteria display feruloyl esterase activity, in their way to hydrolyse the ester bond that connects ferulic acid to polysaccharides (de Oliveira et al., 2014; Wong, 2006).
According to Brenda Enzymes Database, the main producers of feruloyl esterases are belonging to various genera: *Aspergillus, Penicillium, Lactobacillus, Fusarium, Streptomyces, Ruminococcus* etc. (Tables 1 and 2).
The isolated enzymes have various characteristics that differs in accordance with the microbial source: substrate specificity, enzymatic type (A, B, C or D), preferable inductors, hydrolysis of methyl esters, release of free diferulates etc. (Fazary & Ju, 2007; Topakas et al., 2007).
Feruloyl esterases display their activity in a broad range of pH (3-10) and temperature (20-75°C) (Dilokpimol et al., 2016).
The bacterial strains that have displayed high feruloyl esterase activity are included in Table 1.

Table 1. Bacterial strains registered as FAE producers

Bacteria	Reference
Bacillus subtilis	Topakas et al., 2007
B. amyloliquefaciens	Topakas et al., 2007; Wang et al., 2017; Topakas et al., 2007; Fazary & Ju, 2007
Butyrivibrio fibrisolvens	Topakas et al., 2007
B. proteoclasticus	Goldstone et al., 2010; Topakas et al., 2007
Sporotrichum thermophile	Mukherjee et al., 2007; Fazary & Ju, 2007
Streptomyces olivochromogenes	Faulds et al., 1997; Wong, 2006; Donaghy et al., 2000
S. avermitilis	Ferreira et al., 1999; Garcia et al., 1998
S. cinnamoneus	Uraji et al., 2018
Cellvibrio japonicus	Topakas et al., 2007
Lactobacillus acidophilus	Topakas et al., 2007; Xu et al., 2017
L. fermentum	Russo et al., 2016; Topakas et al., 2007; Xu et al., 2017
L. plantarum	Esteban-Torres et al., 2013
Fibrobacter succinogenes	Donaghy et al., 2000; Topakas et al., 2007
Clostridium thermocellum	Blum et al., 2000; Fazary & Ju, 2007; Wong, 2006; Topakas et al., 2007
Pseudomonas fluorescens	Blum et al., 2000; Fazary & Ju, 2007; Ferreira et al., 1999; Wong, 2006; Donaghy et al., 2000;

In order to secrete FAE into the culture medium, scientists have used solid state or submerged fermentations (Fazary and Ju, 2007). Solid state fermentation (SSF) was considered to be the best option for fungal sources, listed in Table 2.
An important step obtaining ferulic acid from fermentations was selecting the best substrates, that will both provide energy and necessary compounds, required for inducing FAE production (Fazary & Ju, 2007).
Some of the best substrates from food and agro-industrial wastes are the ones with high amount of esterified ferulic acid, such as: wheat bran, maize bran, maize fibre, sugar beet pulp, de-starched wheat bran, sugar cane bagasse, corn bran, oat hulls (Fazary & Ju, 2007; Mathew & Abraham, 2005; Camacho-Ruiz et al., 2016; Topakas et al., 2007).

Table 2. Fungal strains registered as FAE producers

Fungi	Reference
Aspergillus awamori	Donaghy et al., 2000; Wong, 2006; Topakas et al., 2007; Fazary & Ju, 2007
A. nidulans	Fazary & Ju, 2007; Topakas et al., 2007
A. flavus	Zhang et al., 2013; Li et al., 2015
A. niger	Ferreira et al., 1999; Wong, 2006; Fazary & Ju, 2007; Topakas et al., 2007; Li et al., 2015
A. oryzae	Wong, 2006; Topakas et al., 2007; Garcia et al., 1998
A. terreus	Li et al., 2015; Topakas et al., 2007
A. nidulans	Fazary & Ju, 2007; Topakas et al., 2007
Penicillium brasilianum	Panagiotou et al., 2007; Topakas et al., 2007
P. chrysogenum	Li et al., 2015
Schizophyllum commune	Faulds et al., 1997; Donaghy et al., 2000
Fusarium oxysporum	Fazary & Ju, 2007; Wong, 2006; Topakas et al., 2007
F. proliferatum	Topakas et al., 2007; Fazary & Ju, 2007
Trichoderma reesei	Topakas et al., 2007

CONCLUSIONS

Lignocellulose is an abundant biomass that still needs more research for its valorization. An economic and environmental approach is its degradation with microorganisms that possess specific enzymatic systems (cellulases, xylanases, ligninases).

Feruloyl esterases are a group of enzymes involved in degradation of lignocellulose by hydrolysing ester bonds and releasing ferulic acid.

Since ferulic acid connects lignin to hemicellulose or hemicelluloses between each other, its displacement is linked to lower recalcitrance of the biomass and a more approachable structure for other enzymes.

In this study, there were presented some of the most used methods for detecting microbial feruloyl esterase activity.

FAE are produced both by bacteria (*Lactobacillus, Ruminococcus, Streptomyces*) and fungi (*Aspergillus, Penicillium, Fusarium).*

Feruloyl esterases have many biotechnological applications such as: food, feed, medical, cosmetic, pharmaceutical, environmental, fuel pulp and paper etc.

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