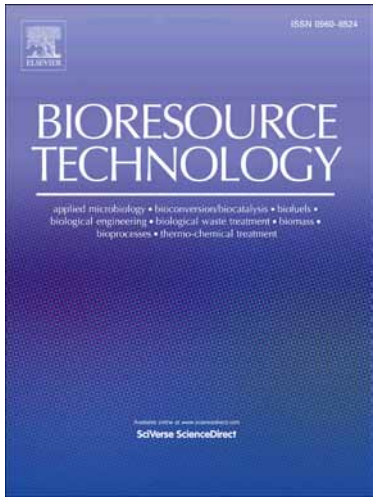
Feruloyl esterases: Biocatalysts to overcome biomass recalcitrance and for the production of bioactive compounds

Dyoni M. Oliveira, Thatiane R. Mota, Bianca Oliva, Fernando Segato, Rogério Marchiosi, Osvaldo Ferrarese-Filho, Craig Faulds, Wanderley D. dos Santos



Review

Feruloyl esterases: Biocatalysts to overcome biomass recalcitrance and for the production of bioactive compounds

Dyoni M. Oliveira, Thatiane R. Mota, Bianca Oliva, Fernando Segato, Rogério Marchiosi, Osvaldo Ferrarese-Filho, Craig B. Faulds, Wanderley D. dos Santos

PII: S0960-8524(19)30077-X
DOI: https://doi.org/10.1016/j.biortech.2019.01.064
Reference: BITE 20941

To appear in: *Bioresource Technology*

Received Date: 12 December 2018
Revised Date: 14 January 2019
Accepted Date: 16 January 2019

Please cite this article as: Oliveira, D.M., Mota, T.R., Oliva, B., Segato, F., Marchiosi, R., Ferrarese-Filho, O., Faulds, C.B., dos Santos, W.D., Feruloyl esterases: Biocatalysts to overcome biomass recalcitrance and for the production of bioactive compounds, *Bioresource Technology* (2019), doi: https://doi.org/10.1016/j.biortech.2019.01.064

Feruloyl esterases: Biocatalysts to overcome biomass recalcitrance and for the production of bioactive compounds

Dyoni M. Oliveira[a1*], Thatiane R. Mota[a1], Bianca Oliva[b], Fernando Segato[b], Rogério Marchiosi[a], Osvaldo Ferrarese-Filho[a], Craig B. Faulds[c], Wanderley D. dos Santos[a*]

[a] Department of Biochemistry, State University of Maringá, Maringá, Paraná, Brazil

[b] Department of Biotechnology, Engineering School of Lorena, University of São Paulo, Lorena, São Paulo, Brazil

[c] Aix-Marseille Université, INRA UMR 1163 Biodiversité et Biotechnologie Fongiques (BBF), 13009 Marseille, France

[1] The authors contributed equally

***Corresponding author:** DMO (dyonioliveira@gmail.com); WDS (wdsantos@uem.br)

Av. Colombo, 5790, Zona 7, Jardim Universitário, Bloco I-89, ZIP 87020-900, Maringá, Paraná, Brazil

Abstract

Ferulic acid and its hydroxycinnamate derivatives represent one of the most abundant forms of low molecular weight phenolic compounds in plant biomass. Feruloyl esterases are part of a microorganism's plant cell wall-degrading enzymatic arsenal responsible for cleaving insoluble wall-bound hydroxycinnamates and soluble cytosolic conjugates. Stimulated by industrial requirements, accelerating scientific discoveries and knowledge transfer, continuous improvement efforts have been made to identify, create and repurposed biocatalysts dedicated to plant biomass conversion and biosynthesis of high-added value molecules. Here we review the basic knowledge and recent advances in biotechnological characteristics and the gene content encoding for feruloyl esterases. Information about several enzymes is systematically organized according to their function, biochemical properties, substrate specificity, and biotechnological applications. This review contributes to further structural, functional, and biotechnological R&D both for obtaining hydroxycinnamates from agricultural by-products as well as for lignocellulose biomass treatments aiming for production of bioethanol and other derivatives of industrial interest.

Keywords: Biorefinery; Carbohydrate esterases; Cell wall; Genome mining; Lignocellulose; Saccharification.

1. Introduction

Feruloyl esterases (FAE; EC 3.1.1.73) are a subclass of carboxylic acid esterases with the capacity to release ferulic acid (FA) and other hydroxycinnamic acids from plant cell walls, plant cytoplasm and synthetic substrates. FAEs are found in bacteria, fungi and plants, with many biotechnological applications, such as: obtaining FA from agricultural by-product wastes and subsequent bioconversion to high added value aromatic compounds, biological delignification of non-woody plants for the paper industry, enzymatic hydrolysis for bioethanol production, improving the digestion of forage plants for ruminants, and as biosynthetic tools to catalyze esterification and transesterification of esters of hydroxycinnamic acids (Furuya et al., 2017; Gopalan et al., 2015; Oliveira et al., 2017).

Ferulic acid (FA) has applications as a food preservative due to its antioxidant and antimicrobial properties; as a therapeutic agent, including anti-inflammatory, antibacterial, antidiabetic and neuroprotective effects; and as a sun protective factor. FA and its derivatives are able to neutralize free radicals, protecting cell membranes and DNA (Paiva et al., 2013). It is also a precursor for synthesis of flavor compounds, such as vanillin and 4-vinyl guaiacol; the microbial biotransformation of FA to vanillin (a major food industry aroma) has been extensively investigated (Furuya et al., 2017). FA is an important factor for lignocellulosic biomass recalcitrance, cross-linking the cell wall polymers. Therefore, the application of FAEs for lignocellulosic biomass deconstruction has a key role in decreasing the biomass recalcitrance to hydrolysis, wherefore lignocellulosic biomass is the most abundant renewable raw material in nature and has the potential to produce biofuels on a scale large enough to replace oil in the mid-term (Linh et al., 2017; Mota et al., 2018).

Biomass recalcitrance is mainly derived from the plant cell wall composition and architecture and it is conferred by different interactions among its components, requiring additional energy inputs for their disruption attack (McCann and Carpita, 2015). The complex architecture of lignocellulose provides a barrier to convert cellulose into fermentable sugars, due to the several factors such as, cellulose crystallinity, accessibility and polymerization, the organization of cellulose microfibrils, hemicellulose polymerization and substitution pattern, lignin content and composition, and the occlusion of the cell wall by lignin-hydroxycinnamate-hemicellulose cross-linking (McCann and Carpita, 2015).

This research review describes the current fundamental knowledge and recent advances in comprehensive mining about biotechnological and biochemical characteristics. Information about several enzymes is systematically organized according to their function,

kinetics, biochemical properties, and substrate specificity. We believe that this mapping of FAEs and their substrate relationship may be a powerful instrument for further functional, biochemical and structural evaluation, as well as biotechnological applications of FAEs to the obtainment of hydroxycinnamic acids from agricultural by-products and improved saccharification of lignocellulosic biomass.

2. Ferulic acid: a polyvalent molecule

2.1. Ferulic acid in plant cell walls and biomass recalcitrance

Ferulic acid is a hydroxycinnamic acid with the systematic name (3-methoxy-4-hydroxy)-3-phenyl-2-propenoic acid or 3-methoxy-4-hydroxy-cinnamic acid (**Fig. 1A**). Produced via the phenylpropanoid pathway, FA and its ester of CoA, FA-CoA, can be seen both as intermediary metabolites towards the formation of monolignols during lignin biosynthesis and as final products of the pathway (Oliveira et al., 2015). Like monolignols and other phenolic compounds, FA presents a polyenic (conjugated) structure that allows the molecule to stabilize a free radical (non-paired electron) produced by UV-light, respiratory chain, peroxidases and other metabolic activities (Bento-Silva et al., 2018). As the ΔG for releasing a free radical is positive, phenylpropanoids stop free radical chain reactions by conserving the radical resonating among their chemical linkages. For this reason, it works as a sunscreen and antioxidant in plants and pharmaceutical products (dos Santos et al., 2008). In plant cell walls, FA plays a key role in inter- and intra-polymer cross-linkage. It may also be covalently linked to lignin through the ether or ester bonds and esterified to polysaccharides (Terrett & Dupree, 2019).

In commelinid monocots — a group of angiosperms including grasses, palms, bromeliads, gingers, and ‘core’ Caryophyllales — ester-linked FA occurs in the cell wall at different and high concentrations (>3.5 mg/g cell wall) according to the specie, organ and tissue, as summarized in **Table 1**. In the primary cell walls of commelinid monocots, including grasses, FA is esterified in the C-5 hydroxyl group of the arabinosyl residue of the arabinoxylan (β-1,4-linked xylopyranosyl backbone) and is more abundant than other groups (Oliveira et al., 2015). FA ester-linked to the arabinosyl residue of arabinoxylan is able to ether-link with lignin or dimerize with other FA-arabinoxylan, cross-linking these cell wall polymers. Such cross-linkages can block the attack of hydrolases, reducing enzymatic hydrolysis efficiency (Oliveira et al., 2015). The production of FA cross-linkages is catalyzed by specific peroxidases that act on FA and FA esters to produce dehydrodimers (e.g. 5-5’ and

8-*O*-4'-dehydrodiferulic acid) cross-linking vicinal arabinoxylan (**Fig. 2**) (Hatfield & Ralph, 1999).

Lignin polymer and feruloylation of plant tissues imposes a barrier to efficient cell wall conversion and negatively impacts digestibility (Ponnusamy et al., 2019). Feruloylation of arabinoxylan is important not only because it leads to cross-linked arabinoxylan in grasses, but also because ferulate may act as a nucleating site for the formation of lignin, and hence linking arabinoxylans to lignin by forming a lignin–ferulate–arabinoxylan complex (Oliveira et al., 2015; Terrett & Dupree, 2019).

Cross-linking of grass cell wall components, especially through FA and diFA esterified to arabinoxylan affects many cell wall properties, such as adherence, extensibility, accessibility and biodegradability. An additional consequence of diFA cross-linkages of arabinoxylan is the increase of recalcitrance with consequent reduction of digestibility of cell wall polysaccharides by glycosyl hydrolases, a limitation for biomass conversion to bioethanol (Oliveira et al., 2015; Perez-Boada et al., 2014).

2.2. Pharmaceutical potential of ferulic acid and derivatives

The therapeutic effect and efficacy of FA are dependent on some properties such as physiological concentration and pharmacokinetics. Generally, in a given meal, people ingest from 180 to 165 mg of FA, which is present in its conjugated and free forms, having low toxicity to the organism and showing a broad variety of biomedical properties: anti-carcinogenic, antioxidant, anti-allergic, anti-inflammatory, antiviral, antimicrobial, and cholesterol-lowering, among others. For that reason, FA is a popular food additive in some countries (Srinivasan et al., 2007).

Due to the observed properties, an extensive study of biological functions and related activities of FA has demonstrated its protective effects against tumor necrosis factor (TNF) and its antimicrobial activities with effects against yeasts and bacteria (Borges et al., 2013). FA has been investigated as an adjunct administrated in cancer chemotherapy due to its capacity to enhance natural immune defenses and gastrointestinal tract movement, protecting the organism against side effects (abdominal discomfort), which limit the treatment (Badary et al., 2006). In addition, the increase of intestinal motility promoted by FA inhibits some intestinal disorders and can act synergistically with antibiotics in the treatment of pathogenic microorganisms such as *Escherichia coli, Helicobacter pylori*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Lysteria monocytogenes*.

The phytochemical-antibiotic combination assays showed a synergistic effect when applied in consort with streptomycin against both pathogenic Gram-negative and Gram-positive bacteria by changing cell morphology and membrane permeability (Shi et al., 2016). FA has mixed antioxidant and pro-oxidant capacities, inducing apoptosis mechanisms in parasites. As an important function, microfilaricidal activity induced by FA culminates in apoptosis and alteration in key antioxidant components, such as glutathione, glutathione S-transferase and superoxide dismutase (Saini et al., 2012). The administration of FA in Wistar rats via intragastric intubation increases protection against lipid peroxidation and decreases the DNA damage due to the improvement in antioxidant performance (Paiva et al., 2013; Sompong et al., 2017).

FA exhibits anti-inflammatory properties, decreasing the levels of compounds produced by macrophages. Diabetic rats supplemented with FA showed a reduction in blood glucose and thiobarbituric acid-reactive substances, and also increases the action of superoxide dismutase and catalase. These results are correlated with the antioxidant capacity of FA, which interacts synergistically with some drugs used in the treatment of diabetes (Nankar et al., 2017). An important function of FA was observed as protective, showing therapeutic action on nephropathy and protective effects in organs such as the brain, kidney, pancreas, intestine and liver by reducing inflammation and oxidative stress (Ren et al., 2017). Additional work has demonstrated that FA increases the levels of monoamine neurotransmitter in the brain and promotes vasodilatation that can be beneficial for the treatment of mood disorders (Chen et al., 2015).

In food science, FA has much utilization due to its intrinsic characteristics in the cell wall together with insolubility of compounds, and can be used in cereal dietary fibers. FA dehydrodimers may be used against *Fusarium graminearum*, a fungus accountable for disease in maize. Besides that, FA shows activity that inhibits the growth of microorganisms and acts as a natural compound that preserves foods, so it has very diversified uses, including preserving oranges, stabilizing soybean oil, inhibiting the oxidation of biscuits, and increasing the viscosity of polysaccharides to obtain gels, and it can be incorporated into materials used to produce drug packages (Silva & Batista, 2017).

3. Classification of feruloyl esterases and genome analysis

Currently, the known FAEs are classified into the Carbohydrate Esterase Family 1 (CE1) within the Carbohydrate-Active Enzymes database (CAZy – www.cazy.org), in

accordance with its amino acid sequences and mode of action (Lombard et al., 2014). Different classification systems have been proposed for FAEs. In this article, we reviewed and explained the different methods used to classify FAEs.

The Crepin's classification system is based in the correlation between previously determined biochemical and functional characteristics and sequence similarities (Crepin et al., 2004). According to Crepin's classification, FAEs can be classified by amino acid sequences similarities into A, B, C, D and a putative E types, and this similarity also correlated with their specificity for synthetic substrates containing hydroxycinnamic methyl esters (methyl ferulate – MFA, methyl *p*-coumarate – MpCA, methyl caffeate – MCA, and methyl sinapate – MSA, **Fig. 1B**), ability to release FA dehydrodimers (especially, 5,5'-diFA, **Fig. 2**) from esterified substrates, and amino acid sequence homology, which also indicates the evolutionary relationship among the microbial FAEs. However, this classification system presents some disadvantages because it is based at the time (2004) on a very small number of confirmed FAEs and sequences. The information of specificity of the enzymes was acquired with different methodologies with natural or synthetic substrates, and unrelated enzymes are part of the same group – e.g. PeFaeA from *Pleurotus eryngii* and AnFaeA from *A. niger*.

According to Crepin's classification, the phylogenetic analysis also suggests that some microbial FAEs do not belong to any kind of type in the previous four classifications. To date, these enzymes are predominantly bacterial in origin, suggesting that fungal and bacterial FAEs are encoded different from each other. Therefore, these enzymes are classified in the putative type E sub-class. This fifth sub-class comprises *Orpinomyces* sp. OspFaeA, *Clostridium thermocellum* XynZ, *Ruminococcus* sp. RspXyn1, *R. flavefaciens* RfXynE and *R. albus* RaXynB. Nevertheless, members of this sub-class may only be identified based on their primary amino acid sequence identity. No further correlation could be established due to the lack of comparable enzyme activity data (Crepin et al., 2004). This classification has been used by other researchers in characterizing their FAES. For example, the cinnamoyl esterase from *L. acidophilus* F46 is classified into the putative type E, indicating that the functional classification of the microbial FAE based on fungal origin is not enough to describe it according to Crepin's classification (Kim & Baik, 2015).

As a further refinement for FAEs classification, Benoit and coworkers provided an elegant system based on the phylogenic analysis of known fungal genomic sequences expanding the comprehension of known FAEs (Benoit et al., 2008). Based on Benoit's classification system, seven subfamilies (SF 1 to 7) of candidate FAEs were identified in many fungal genomes, containing three biochemically characterized types of FAEs (types A,

B and C), excluding those esterases classified previously as type D and all bacterial FAEs. Members of the genus *Aspergillus* are present in the subfamily 1, 3, 4, 5, 6 and 7. Subfamily 7 is restricted to FAEs genes from *A. niger*, *A. oryzae* and *A. terreus* based on the primary dataset these authors had access to. The same is almost true for subfamily 1 that contains FAE genes from *A. fumigatus, A. niger, A. oryzae* and *A. nidulans*. However, this subfamily also includes the FAE gene from *Talaromyces stipitatus* as a member and is closely related to subfamily 2, while subfamily 7 is very distant from all the other subfamilies.

Udatha and coworkers extended the classification of FAEs further by proposing a novel classification system, grouping for the first time FAEs from three kingdoms – fungi, bacteria and plant. The classification resulted in 12 distinct families, which have the capability of acting on a large range of substrates for cleaving ester bonds and synthesizing high-added value molecules through esterification and transesterification reactions (Udatha et al., 2011). In this classification system, 365 putative FAE-related sequences of fungi, bacteria and plant origin were collected and clustered into distinct groups based on amino acid composition, protein secondary structure, and physicochemical composition descriptors derived from the respective amino acid sequence. The 12 proposed families were validated applying a combination of prediction tools with experimental data. Unfortunately with this, FAEs share a high sequence identity with other serine proteases, such as acetyl esterases, proteases and lipases, and so a sequence-based only classification is difficult to predict without corresponding biochemical data confirming that these sequences do correspond to actual FAEs.

Recently, the groups of de Vries and Hildén have proposed another classification for fungal FAEs considering both phylogeny and substrate specificity (Dilokpimol et al., 2016). In this latest classification, 13 sub-families are proposed together with an unclassified group, demonstrating the limitations in grouping both bacterial and fungal FAEs together. However, according to this classification, only some FAEs from sub-families 5 and 6 are grouped in CE1 family of CAZy database (Udatha et al., 2011). More recently, the same research groups performed a phylogenetic analysis that divided fungal FAEs members of CE1 family into five subfamilies, this upgrade in the last classification system was able with the identification and characterization of a novel AtFaeD from *A. terreus* (Dilokpimol et al., 2016).

With the expansion of microbial genome sequencing, it has been revealed that organisms contain a multiplicity of putative enzyme-encoding genes. Multiple predicted FAE-encoding genes have thus far been identified in fungi, which will allow more FAEs to be heterologously expressed and characterized soon. While some fungi, such as *Trichoderma*

reesei, do not contain FAE-encoding sequences (Martinez et al., 2008), other species in the *Trichoderma* genus do contain putative FAEs in their genome (Dilokpimol et al., 2016). Thirteen predicted FAEs where identified in the genome of *A. oryzae* belonging to six subfamilies of the Udatha's classification. These sequences were subsequently modeled for structural analysis and three sequences cloned and expressed in *Pichia pastoris* (a system well-disposed for esterase expression), and their substrate specificity was determined. Furthermore, 37 putative FAEs were identified in the basidiomycetes *Moniliophthora roreri*, *Auricularia subglabra* and *Agaricus bisporus* var *bisporus,* and predicted genes encoding FAE were identified in the genomes of several ascomycetes such as *A. flavus* with 16 genes, 29 in *A. niger*, 16 in *Chaetomium globosum*, and 11 in *Fusarium graminearum* (Dilokpimol et al., 2016). As these authors pointed out, however, a genome sequence prediction is not always accurate, as pseudogenes and non-FAE related enzymes, such as tannases, acetyl esterases, and others, could be included. Similarly, gene cloning followed by protein expression and characterization are essential to determine the ability of a microbe to produce multiple FAEs. More recently, Dilokpimol and coworkers confirmed the ability of the genome mining strategy to identify eligible candidates for fungal FAE encoding genes for related biotechnological applications, by demonstrating that 20 out of 27 putative fungal FAEs possessed esterase activity (Dilokpimol et al., 2018).

4. Biochemical properties of microbial FAEs

Since their first identification in the 1980s and purification in 1991 (Faulds & Williamson, 1991), more than 80 FAEs with different molecular masses, substrate preferences, isoelectric points, and optimum reaction conditions have been characterized from microbial sources. Microbial FAEs from different sources present a broad range of biochemical properties and kinect values, as summarized in **Table 2**. The biochemical characteristics of FAEs show significant variation in molecular weight (18.5 – 210 kDa), isoelectric points (3.0 – 9.9), optimum pH (3.0 – 10.0) and temperature (20 – 75 °C). Based on the biochemical properties of characterized FAEs (**Table 2**), we organized the data for molecular mass, optimum temperature, pH and isoelectric point for better understanding and to contribute to further functional studies (**Fig. 3).** According to relative frequencies and medians, FAEs frequently have a molecular mass of 36 kDa (**Fig. 3A**), optimum temperature at 50 °C (**Fig. 3B**), and pH at 6.5 (**Fig. 3C**), and they have acidic p*I* values suggesting a high proportion of negative amino acids (**Fig. 3D**).

FAEs display a common fundamental mechanism having at their active site a catalytic triad consisting of a serine (Ser), a histidine (His) and a carboxylic acid, mainly aspartic acid (Asp) or glutamic acid**.** In the classical catalytic triad, the protonation states or the net charges of Asp and His residues are very important for maintaining the activity of FAEs (Udatha et al., 2012). In this sense, the FAE-catalyzed reaction is very similar to the hydrolytic action of serine proteases, lipases and other esterases, involving a covalent acyl-enzyme intermediate (Uraji et al., 2018). As we presented in **Fig. 3C**, FAEs are optimally active at pH 6.5, suggesting a clear importance of pH dependence by FAEs, His acts as a general acid-base catalyst, and Asp neutralizes the charge that forms on His during the catalytic process (Udatha et al., 2012).

As described above, each FAE has its own specificity, releasing specific hydroxycinnamic acids such as FA, *p*-coumaric, sinapic or caffeic acids from their esters. Several methods have been developed for FAE activity determination with natural and synthetic substrates (Ramos-de-la-Peña & Contreras-Esquivel, 2016). Numerous model substrates including methyl and ethyl esters of hydroxycinnamates have been evaluated as monoferuloylated 4-nitrophenyl glycosides and natural substrates such as FAX (2-*O*-[5-*O*-(*trans*-feruloyl)-β-L-arabinofuranosyl]-D-xylopyranose) and FAXX (*O*-[5-*O*-(*trans*-feruloyl)-α-L-arabinofuranosyl]-(1→3)-*O*-β-D-xylopyranosyl-(1→4)-D-xylopyranose) (Hunt et al., 2017; Topakas et al., 2012a).

The kinetic values, K_m, k_{cat} and k_{cat}/K_m (**Table 2**) are affected by structural differences in the substrate, such as: (1) the type of substitutions on the phenolic ring, as hydroxyl or methoxyl groups; (2) the number of substitutions on the phenolic ring; (3) the distance between the ester bond and the phenolic ring; (4) the presence or absence of unsaturation in the aliphatic chain; and (5) the number of sugar residues linked to the phenolic acid (Hunt et al., 2017).

An important proof-of-principle demonstrated that FAEs can hydrolyze nonpolar ferulic acid esters (Schär et al., 2016). A systematic evaluation of the activity of FAEs from different classes (AnFaeA, MtFaeB, CtFae and RoFae) on nonpolar *n*-alkyl ferulates was carried out to evaluate if microbial FAEs are capable of hydrolyzing naturally occurring *n*-alkyl ferulates. A decrease in K_m and k_{cat} is correlated with decreased substrate polarity for FAEs. This led to the conclusion that for FAEs, nonpolar ferulic acid esters such as long-chain *n*-alkyl ferulates are very poor substrates.

5. Heterologous expression of FAEs by microbial systems and protein engineering

Most well characterized FAEs have been cloned and expressed in heterologous hosts in order to a) make them suitable for industrial applications by increasing their thermal stability, protease resistance, and pH stability; b) understand structure-function relationships; c) delineate the role of particular amino acid residues; d) increase production; and e) purify enzymes in single step with high yield and specific activity (for example, an *E. coli* based expression system is suitable for protein engineering and high-throughput structural analysis) (Zhang et al., 2012; Zhang & Wu, 2011). Previous studies have predominantly focused on AnFaeA isolated from *A. niger*. The enzyme has been cloned and successfully expressed in *E. coli*, *P. pastoris*, and *A. niger* expression systems.

Increasing the repertoire of cloned FAEs, Wu and colleagues performed studies with two FAEs from the myxobacterium *Sorangium cellulosum*, ScFae1 and ScFae2, which were cloned into a pSD80 vector and expressed in *E. coli* as a soluble fraction, followed by three purification steps (DEAE, Butyl-S and Superdex 75 column) (Wu et al., 2012). Wong and coworkers isolated the FAE gene from microflora of a cow's rumen (RuFae2), cloned into *E. coli* and expressed in active form (Wong et al., 2013). The enzyme RuFae2 had the amino acid sequence closely related to the primary structures of bacterial FAEs from *Prevotella oris* C735 (41.9% identity) and *L. johnsonii* (47% identity). The *E. coli* expression system has advantages and disadvantages. An example of the disadvantage using *E. coli* BL21 (DE3) cells, the cDNA of AcFae from *A. clavatus* was cloned into pET28a vector and overexpressed as an insoluble fraction (Damásio et al., 2013). The inclusion bodies containing the protein had to be denatured and purified by affinity chromatography, and after the refolding, the extract was concentrated and the enzyme was purified by gel filtration chromatography, which resulted in a small fraction of purified enzyme showing activity. On the other hand, XynZ from *C. thermocellum* (containing domains corresponding to xylanase and FAE) was well-expressed in *E. coli* BL21 (DE3) as a soluble fraction and purified by two steps — first, immobilized metal ion affinity chromatography, followed by a size exclusion chromatography, which resulted in a high fraction of purified enzyme (Mandelli et al., 2014).

Using a *P. pastoris* GS115 expression system, Yin and coworkers investigated the effects of disulfide bridges on the thermostability of AuFaeA from *A. usamii* introducing an extra disulfide bridge to, or by eliminating each native disulfide bridge in, the protein sequence (Yin et al., 2015a). The experimental results confirmed that the disulfide bridges contribute significantly to the thermostability of AuFaeA. This same group improved the thermostability of AuFaeA by iterative saturation mutagenesis of Ser33 and Asn92 (Yin et al.,

2015b). The study revealed that the best variant Ser33E/Asn92-4 produced a T_m value of 44.5 °C (39.8 °C in WT), the half-life of 198 min at 50 °C, corresponding to a 3.96-fold improvement compared to the wild-type. Further, the best Ser33 variant Ser33-6 was thermostable 32 min longer than wild-type at 50 °C with a half-life of 82 min.

The substrate specificity of FAE involves interaction between the substitutions on the phenolic ring of the hydroxycinnamates with the residues within the active site of FAEs. For AnFaeA, it was shown that the OH and OCH_3 of FA interact with the hydroxyl groups of Tyr80 (Faulds et al., 2005). When the importance of the polar and aromatic residues in the active site of AnFaeA was investigated, it was shown that these residues are involved in binding, as measured by K_m, but also the specificity, as replacement of Tyr80 and Trp260 with smaller residues such as valine or serine resulted in a broadening of the substrate specificity of this esterase. Mutations in the lid/flap region of AwFaeA from *A. awamori*, especially a Tyr to Phe or Ile mutation, changed the hydrophobicity of the region and hence the substrate discrimination of this esterase towards long-acyl chain naphthyl esters, as has been found for lipases, as well as influencing its optimum pH (Koseki et al., 2005). When a Phe to Ile mutation on a corresponding residue was constructed in a *Thermomyces lanuginosa* lipase, the enzyme obtained significant FAE activity, suggesting that the mutant adopted an open conformation to accommodate FA-type substrates into the active site in the aqueous phase (Andersen et al., 2002).

Directed evolution was applied to improve the thermostability of AnFaeA (Zhang et al., 2012) and the dimeric EstF27 from a soil metagenomics library (Cao et al., 2015). Twelve residues in AnFaeA, four being surface exposed and eight being in the inner part of the enzyme, were identified after directed evolution to be beneficial to the thermostability of AnFaeA. After two rounds of directed evolution, the thermostability of the mutated EstF27 at 50 °C was improved over 3000-fold, and a 1.9-fold higher catalytic efficiency and a 2-fold improvement of FA release from wheat bran was obtained as a result of six amino acid substitutions, mainly associated with the dimer interface, leading to the formation of a new disulphide bond and a general increase in hydrophobicity of the protein.

The EstF27 isolated from a soil metagenomics library and overexpressed in *E. coli* displays low thermostability at temperatures higher than 50 °C but was able to release FA efficiently from wheat bran (Sang et al., 2011). Furthermore, EstF27 had its thermostability improved by two rounds of random mutagenesis (Cao et al., 2015). The mutations promoted an increase in the optimal temperatures to 60 °C (mutant M4) and 65 °C (mutant M6), which correspond to 20 °C and 25 °C higher than that shown by the wild-type. In addition, the

storage stability of wild-type and mutant M6 was also assessed. After storage at 25 °C for 150 days, the activity of M6 remained constant (100%), whereas only residual activity remained with the wild-type (21.2%). The kinetic values also increased; the k_{cat}/K_m value of M6 at its optimal temperature of 65 °C is about 1.9-fold higher that of wild-type. High-throughput screening (HTS) was successfully applied for the generation of 30,000 mutants of MtFae1a from *M. thermophila,* followed with their screening for selecting the variants with higher activity than the wild-type enzyme (Varriale et al., 2018)

6. Three-dimensional structures

Only a few crystal structures of FAEs have been solved to date, which makes our understanding of the residues involved in specific substrate interactions for the different classes of FAEs rather limited. Structural information is important if one decides to improve or change catalytic function for biotechnological applications. The structures of the two FAE modules of *C. thermocellum* XynY and XynZ were the first to be solved (Prates et al., 2001; Schubot et al., 2001), each displaying the canonical eight-strand α/β fold of lipases/esterases. This fold is also called the TIM-barrel and forms the active site cleft at the carboxy-terminal of the sheet. A lid, analogous to lipases, confines the active site cavity with a loop that confers plasticity to the substrate-binding site.

Some FAEs have sequences and structures related to lipases, showing the same serine active-site motif in a α/β-hydrolase fold, which consists of nine β-sheet core surrounded by five α-helices and two additional β-strands indicative of enzymes capable of hydrolyzing synthetic ferulate dehydrodimers (**Fig. 4A-C**). Many FAE models have been constructed based around sequence identity to lipases where crystal structure was unavailable. It is believed that a functional shift following a duplication event was responsible for the neofunctionalization of fungal FAEs from ancestral lipases (Levasseur et al., 2006; McAuley et al., 2004). On the other hand, unlike FAEs, lipases show two conformational states with an opened or closed active site, while FAEs have only the first configuration. **Fig. 4** shows the closed conformation in the structures of AnFaeA from *A. niger* with a lipase from *T. lanuginosa* in its open conformation linked with the respective substrates in the catalytic cleft (**Fig. 4D-F**). Lipases are lipolytic carboxyl ester hydrolases with the ability to hydrolyze water-insoluble esters, releasing long-chain fatty acids, whereas esterases act on water-soluble esters bearing short-chain acyl residues (Romano et al., 2015).

The classical catalytic triad at the core of the active sites of these two esterases and that of AnFaeA (**Fig. 4**) were identified in the hydrophobic binding pocket (Hermoso et al., 2004; McAuley et al., 2004; Uraji et al., 2018). In addition, biochemical studies displayed the importance of the position of the ester-linkage and the length and composition of the sugar-sugar moieties esterified to FA in the catalytic performance of AnFaeA (Faulds et al., 2005). To date, the carbohydrate part of the FA-arabinoxylooligosaccharides used in co-crystallization studies has never been visualized. This lack of visualization of the carbohydrates in the crystal structures suggests a loose interaction between the enzyme binding residues and the carbohydrate, perhaps reflecting the heterogeneity of the arabinoxylan polymers and a more specific, tight binding to the phenolic ring. Structural models of the MtFae1 were constructed, and this esterase resembled a lipase-type structure with a small cap-like domain over the active site, which is postulated to influence substrate specificity (Topakas et al., 2012b). While MtFae1 resembles lipases, the structure of *A. oryzae* AoFaeB indicated that this esterase exists as a dimer, contains a novel CS-D-HC motif at the catalytic site, and is more closely related to tannases than lipases (**Fig. 4**). Presenting a unique lid structure, such proteins display no significant structural similarities to any protein in the Protein Data Bank (Suzuki et al., 2014).

The three-dimensional structure of AoFaeB from *A. oryzae* is composed of two regions that are identified as catalytic domains containing the Ser-His-Asp triad (**Fig. 5A dashed circle**) and the lid domain which covers the active site (**Fig. 5 blue region**) (Suzuki et al., 2014). The lid domain contains a calcium ion coordinated by water and five amino acids (Asp272, Asp 275, Ala277, Asp 279 and Ile281), which are far from the active site and involved in lid domain stabilization (**solid circle in Fig. 5A**). The catalytic domain shows α/β-hydrolase fold, and in an interface between these domains and the lid domain, the active center of the AoFaeB, which is covered by the lid forming the substrate pocket of the enzyme (**Fig. 5B and D**).

7. Identification of glycosylation sites

It is desirable that enzymes used in industrial processes resist non-physiological conditions such as extreme pH and temperature. Glycosylation has long been identified as a key point in the thermal stability of glycoproteins, representing a promising target for protein engineering efforts. Glycosylation is a post-translational modification with biological functions in recognition, signaling and thermostability of enzymes. Secreted enzymes are

often covalently *N*-linked and *O*-linked to glycosides at specific sites. *N*-glycosylation generally attaches a branched mannose or a single *N*-acetylglucosamine residue to the β-amide group of an asparagine residue, while *O*-glycosylation attaches the carbohydrate (generally one to three mannose residues) to the β-hydroxyl group of a serine or threonine residue (Zhang et al., 2015).

In an investigation of the effect of glycosylation on protein mass, interestingly, *P. sapidus* PsEst1 presents a protein band in SDS-PAGE 10 kDa higher than the theoretical molecular mass, which was 59.4 kDa, as shown in **Table 3** (Kelle et al., 2016). The amino acid sequence analysis of PsEst1 reveals four potential *N*-glycosylation sites that after being removed from the purified PsEst1 using endo-β-*N*-acetylglucosaminidase H, the deglycosylated enzyme presents 60 kDa by SDS-PAGE. AtFaeA from *A. terreus* has two potential *N*-glycosylation sites and 53 potential *O*-glycosylation sites, which is more than the 46 and 49 potential *O*-glycosylation sites of *A. flavus* AfFaeA and AnFaeA, respectively (Zhang et al., 2015). SDS-PAGE protein bands of AtFaeA have around 40 and 37 kDa, and the purified protein treated with endo-β-*N*-acetylglucosaminidase H resulted in a major protein band of 35 kDa. The AnFaeA and *A. tubingensis* AtFaeA enzymes are very acidic proteins, showing a p*I* of 3.3 and molecular mass of 28 kDa for both proteins. However, the SDS-PAGE indicates an apparent molecular mass of 36 kDa, due to a high amount of glycosylation in its structure (de Vries et al., 1997; Faulds & Williamson, 1994). The enzyme AnFaeB has 18 *N*-glycosylation sites and the electron-density maps in this region indicated no additional carbohydrate residues attached to the *N*-acetylglucosamine residue (de Vries et al., 1997; McAuley et al., 2004).

The *N*-glycosylation site in AnFaeA coupled with the high ratio of polar residues in this region stabilizes the 'lid' of the esterase in an open configuration, thus conferring an esterase character rather than an interfacial-active lipase activity (Hermoso et al., 2004). This raises the question of the actual importance of glycosylation in the thermal stability of AnFaeA. The thermal stability of four molecular forms of AnFaeA — native, refolded, glycosylated, non-glycosylated — was evaluated, demonstrating that AnFaeA produced in *A. niger* (melting point temperature of 56 °C) is more heat resistant than AnFaeA produced in *E. coli* (melting point temperature of 52 °C) recovered by inclusion bodies and refolded without glycosylation (Benoit et al., 2006a).

The crystallographic structure of AnFaeA in its native form revealed a fungal *N*-glycosylation pattern linked to Asn79. The mature protein NcFae-I of *N. crassa* contains a molecular mass estimated by SDS-PAGE 6 kDa higher than the calculated molecular mass of

29.2 kDa, the *N*-glycosylation was confirmed by electrospray ionization-mass spectrometry, which revealed a molecular mass of 35.04 kDa (Crepin et al., 2003a) . AnFaeB and TsFaeC contain 521 and 530 amino acids and calculated molecular masses of 55.5 kDa and 55.34 kDa, respectively. Nevertheless, the native proteins are glycosylated, yielding proteins with molecular masses of 74 kDa and 65 kDa, respectively. Deglycosylated AnFaeB has a molecular mass of 60 kDa by SDS-PAGE analysis (Crepin et al., 2003b; Garcia-Conesa et al., 2004) .

A study describing a novel feruloyl esterase from *A. niger*, AnFaeC, found that unlike AnFaeA and AnFaeB, it did not show a decrease in molecular mass after deglycosylation, maintaining the molecular weight of 30 kDa (Dilokpimol et al., 2017). In contrast, the PpFaeA and PcFae-1 from *P. purpurogenum* and *P. chrysogenum*, respectively, showed slight reduction of molecular mass from 35 to 32 kDa for PpFaeA and from 62 to 60 kDa for PcFae-1 with the deglycosylation treatment (Oleas et al., 2017; Sakamoto et al., 2005). Zhang and coworkers (2013) and Topakas and coworkers (2012b) did not confirm the glycosylation of AfFaeA from *A. flavus* and MtFae1 from *M. thermophila,* but compared it with the theoretical molecular mass and suggested the enzymes may be glycosylated due to the potential N-glycosylation sites found out. This analysis was performed based on the presence of Asn–Xaa–Thr/Ser sequons, which are characterized as an oligosaccharide acceptor and a prerequisite for N-glycosylation. A great extent of glycosylation was observed in the studies of Rumbold et al. (2003) and Shin and Chen (2007) of feruloyl esterases AN1772.2 (from *A. nidulans*) and ApFae (from *A. pullulans*). The AN1772.2 glycosylated contained more than double the molecular mass of deglycosylated enzyme and the ApFae showed about 48% of glycosylation influence on the molecular mass value (**Table 3**).

The *A. oryzae* enzymes, AoFaeB and AoFaeC, have an apparent relative molecular mass of 61 and 75 kDa, respectively, on SDS-PAGE. After *N*-deglycosylation, both proteins had a relative molecular mass of 55 kDa, suggesting that both enzymes are *N*-glycosylated. Thirteen and ten potential *N*-glycosylation recognition sites are present in the AoFaeB and AoFaeC sequences, respectively (Koseki et al., 2009). Previously, Koseki and colleagues also reported that *N*-glycosylation is important for the thermostability and protein folding of the AwFaeA from *A. awamori*, similar to the results reported for an FAE of *A. niger* (Koseki et al., 2006). *N*-linked glycosylation motif (Asn79-Tyr-Thr) was found in the sequence of FAEs from Aspergilli enzymes (Benoit et al., 2006a). Understanding the role of *N*-linked oligosaccharides located in the flap region made it possible to clarify the biochemical properties of AwFaeA from *A. awamori* expressed in *P. pastoris.* The analysis of removed *N*-

linked glycosylation recognition sites by site-directed mutagenesis demonstrated that Asn79 replaced with Ala79 or Gln79 had lower activity than glycosylated wild-type AwFaeA (Koseki et al., 2006). Both mutant enzymes exhibited a significant decrease in hydrolysis rate and efficient catalysis. The wild type showed a K_m of 0.26 mM and k_{cat}/K_m of 588 s^{-1} mM^{-1}, the mutant Ala79 values were K_m 0.66 mM and k_{cat}/K_m 145 s^{-1} $mM^{-1,}$ and mutant Gln79 values were K_m 0.28 mM and k_{cat}/K_m 73 s^{-1} mM^{-1}. These data suggest that the glycan chains affect substrate discrimination and contribute to the stabilization of the flap in its open conformation in the wild-type Asn79.

8. Releasing FA and FA dehydrodimers from plant cell walls

Monomers and dimers of FA are important structural components of complex plant cell walls. FAEs were applied to release FA and diFA from numerous agro-industrial by-products, as wheat bran, maize bran, maize fiber, brewer's (or barley) spent grain, corn stalk, sugar beet pulp, coastal bermudagrass, oat hulls, sugarcane bagasse, jojoba meal, wheat straw, and elephant grass. The cleavage of a linear xylan backbone yields short feruloylated oligosaccharides (Oliveira et al., 2016). Specific cell wall degrading enzymes such as xylanase are required to solubilize part of the cell wall structure by forming short feruloylated xylooligosaccharides. Then, FAE may act on these feruloylated compounds to release FA. In turn, the removal of FA residues from feruloylated xylooligosaccharides makes them again more accessible for further hydrolysis by xylanase and other degrading enzymes (Long et al., 2018).

Faulds and coworkers were able to release hydroxycinnamic acids from brewers' spent grain by applying a *Humicola insolens* commercial enzyme cocktail containing FAE activity, the reaction releases of 76, 40, 71 and 73% of FA, *p*-coumaric, 5,5'-diFA and 8-*O*-4'-diFA, respectively, in comparison with the total released in alkali (TRA) (Faulds et al., 2004). Benoit et al. (2006b) studied the ability of AnFaeA and AnFaeB to release hydroxycinnamic acids, such as FA, *p*-coumaric, and caffeic acids from coffee pulp, maize bran, apple marc and sugar beet pulp. AnFaeB releases 100% and 83% of caffeic acid from coffee pulp and apple marc, respectively and 73% and 34% of *p*-coumaric acid, respectively, while AnFaeA does not show substantial effects on these biomasses. On the other hand, 40% of FA is released with AnFaeA compared to 8% with AnFaeB from autoclaved maize bran.

Some microbial FAEs release dehydrodimers and, therefore, can potentially break down xylan-lignin crosslinks. *P. fluorescens* PfXylD and AnFaeA, alone or in concert with a

xylanase, release 5-5′-diFA and 8-*O*-4'-diFA from barley and wheat cell walls (**See Fig. 2**). AnFaeA is able to release 5-5′-diFA from brewers' spent grain and wheat bran (Faulds et al., 2006). The type of xylanases used in synergy with FAEs affects the level and form of FA released. Glycoside hydrolase family 11 xylanases (GH11) are more efficient at releasing FA, whereas family 10 xylanases (GH10) are more effective at releasing diFA (Faulds et al., 2006).

FaeLac from *Lactobacillus acidophilus* K1 hydrolyzes brewers' spent grain and releases different amounts of HCA, when compared with TRA: FA (2.1%), *p*-coumaric acid (2.9%), sinapic acid (2.8%), and caffeic acid (3.7%) (Szwajgier et al., 2010). AcFae from *A. clavatus* is able to release FA and *p*-coumaric acid from sugarcane biomass and insoluble wheat arabinoxylan (Damásio et al., 2013). The enzymatic hydrolysis of wheat arabinoxylan releases up to 85% of the alkaline extractable *p*-coumaric acid and 2-fold more FA than TRA, after 15 h of incubation. In turn, AcFae exhibited only 37% and 7% efficiency for releasing FA and *p*-coumaric acid from sugarcane bagasse, respectively. Cao et al. (2015) conducted a detailed study using EstF27 from the soil metagenomics library and EstF27 thermostable mutant to hydrolyze wheat bran for 10 h at 40 °C. The EstF27 was able to release 17.7% and the mutant 21.6% of the TRA from wheat bran. At 65 °C, the mutant released 36.8% of TRA FA but the EstF27 was completely denatured after 10 h.

9. Synergism between FAEs and hemicellulases

The maximum bioconversion of lignocellulosic material requires the action of efficient cellulolytic enzymes in synergy with hemicellulases and auxiliary enzymes. The utilization of hemicellulose sugars is essential for the efficient and cost-effective conversion of lignocellulosic biomass to biofuel (Braga et al., 2014; Li et al., 2014).

The degree of synergy or synergism measures the ability of two or more enzymes to cooperate in each other's action upon a substrate (Dyk & Pletschke, 2012). The synergism is also dependent on the properties of the substrate, the specific enzymes and other experimental conditions, such as enzyme loading in the reaction mixture (Jia et al., 2015). FAEs exhibit strong synergistic relationships with endo-hydrolases and debranching enzymes, such as xylanases, arabinanases, galactanases, mannanases, polygalacturonases, and rhamnogalacturonases (Mandalari et al., 2008; Segato et al., 2014; Xue et al., 2017). FAEs have been employed to improve biomass degradation because they disrupt the lignin-

cellulose-hemicellulose network, increasing the accessibility of cellulases and hemicellulases to their respective substrates (Debeire et al., 2012).

FA is efficiently released from a wheat bran preparation by AnFaeA plus xylanase. AnFaeA released 24-fold more FA from wheat bran when xylanase is added in the incubation. AnFaeB released 1% FA from sugar-beet pulp, while the addition of arabinanase and arabinofuranosidase in the reaction mixture improved the release of FA to 12% (Kroon and Williamson, 1996). AnFaeA and AnFaeB are involved in the degradation of pectin and xylan substrates, but they have opposite preferences for these polysaccharides (de Vries et al., 2002). AnFaeB is most active towards sugar-beet pectin, whereas AnFaeA is most active in wheat arabinoxylan, demonstrating a clear substrate specificity of AnFaeB for feruloylated pectin oligosaccharides. The incubation of AnFaeB with arabinofuranosidase and β-galactosidase (both enzymes from *A. niger*) increases the hydrolysis of sugar-beet pectin, though the concomitant incubation of AnFaeA and AnFaeB do not result in an increment in the amount of FA released compared with AnFaeB alone (de Vries et al., 2002).

Topakas and coworkers observed the maximal release of FA (33% of TRA) from wheat bran, applying 0.4 U/g of *M. thermophila* MtFae with 500 U/g xylanase within 1 h of reaction time (Topakas et al., 2004). *E. coli*-expressed *S. cellulosum* ScFae1 and ScFae2 were purified and applied in triticale bran hydrolysis. ScFae2 appeared to be more efficient releasing FA from triticale bran (Wu et al., 2012). The experiments with 0.45 U/ml of ScFae2 incubated with 5.3 U/ml of *T. viride* xylanase demonstrated that 3 h of hydrolysis resulted in a release of 96% of FA from triticale bran. The application of blends of enzymes produced by the fungi *T. reesei* and *A. awamori* efficiently hydrolyzed steam-pretreated sugarcane bagasse (Gottschalk et al., 2010). *A. awamori* produced FAE, which acts synergistically with cellulolytic–xylanolytic enzymes, enhancing the effectiveness of the cellulase and xylanase enzyme blends. Li et al. (2011) reported the production of three FAEs from *Cellulosilyticum ruminicola* H1, FaeI, FaeII and FaeIII. The addition of FaeI and FaeII to xylanase from *T. lanuginosus* increased 37% and 27%, respectively, the amount of reducing sugars released from maize cob in 6 h of incubation at 38 °C. Likewise, FaeI elevated cellulase activity by 17%, while FaeII and FaeIII reduced it by 34%. In turn, both cellulase and xylanase enhanced the activities of the three FAEs between 2 to 40%, displaying the highest degree of synergy (1.5) with FaeIII.

The addition of RuFae2 in reaction mixtures of endoxylanase GH10 from *Cellvibrio mixtus* increased 6.7-fold the release of FA from wheat bran and 2.7-fold from wheat-insoluble arabinoxylan (Wong et al., 2013). Zhang et al. (2015) conducted a thorough study

using AtFaeA from *A. terreus* with xylanase from *A. niger* to hydrolyze different particle sizes (20, 40, 80, 100 mesh) of corn stalk and corncob for 1 h at 50 °C. The content of reducing sugars produced from corncob is about 2-fold higher than from corn stalk, and the production of them increased along with the particle size of corncob and corn stalk. Recently, our research group evaluated the synergistic effect of AcFae from *A. clavatus* and a commercial xylanase preparation on sugarcane bagasse hydrolysis at 30 °C for 24 h (Oliveira et al., 2016). The treatment resulted in a significant increase in the amount of reducing sugars (1.97-fold) released from sugarcane bagasse, an agricultural residue containing a high concentration of FA. Although the treatment with AcFae plus xylanase released only 7.7% of TRA, the enzymes show a high degree of synergy, 5.1.

Evaluating the simultaneous cooperation of AnFaeA and xylanase GH11 (AnXyn11A) in hydrolyzing wheat bran for the co-production of FA and xylooligosaccharides, Wu and coworkers applied AnFaeA (100 U) and AnXyn11A (0 to 1000 U) simultaneously, and the FA released was greatly enhanced from 16.8% to 70% as the activity of AnXyn11A increased from 0 to 300 U, and xylooligosaccharide yield almost doubled in the optimum level of enzyme addition (Wu et al., 2017). On the other hand, AnFaeC from *A. niger* in synergy with xylanase (*T. lanuginosus*) releases FA and *p*-coumaric acid from wheat arabinoxylan and wheat bran, but did not show cooperative effect with endopolygalacturonase, rhamnogalacturonan hydrolase and rhamnogalacturonan acetyl esterase (Dilokpimol et al., 2017). The proof-of-principle was able to demonstrate the differential effect of sequential or co-incubation treatment with FAE and xylanase to release FA from lignocellulosic substrates. The releasing of FA from wheat arabinoxylan was 11-fold more efficient when the substrate was sequentially pre-treated with xylanase followed with AtFaeD than the enzymes co-incubated together (Mäkelä et al., 2018). Besides, the production of xylooligosaccharides from wheat arabinoxylan was successfully improved by 27% to 30% with the treatment with AtFaeD from *A. terreus* and commercial xylanase.

Several groups are currently working to understand precisely why FAEs and debranching enzymes respond differently towards the feruloylated polysaccharides. This phenomenon indicates that FAE isoenzymes may target different substrates in a complementary manner, contributing to the efficient degradation of diverse plant biomass. Future investigations should therefore center upon more complex enzymatic cocktails, or mixtures of cocktails, that accurately reflect the complexity of plant cell walls.

10. Immobilization and influence of organic co-solvents on the FAE activity

In industry, immobilization is often employed to stabilize enzymes and facilitate their reuse, reducing costs. Immobilized enzymes can be used for the *de novo* synthesis of bioactive compounds and platform chemicals, and in the development of nanomaterials for biomedical applications. The immobilization of FAE has been studied for such applications. Monocomponent FAEs have been immobilized on magnetic Fe_3O_4 nanoparticles, leading to an increase in optimal temperature from 45 ^oC to 55 ^oC and temperature stability, retaining 52.4% of its initial activity for the release of FA from insoluble wheat bran after 5 cycles (He et al., 2015). Supermagnetic Fe_3O_4@Au core-shell nanoparticles were used to immobilize a putative FAE, PhEst, with a 2-fold increase in activity compared to the free enzyme (Parracino et al., 2011). It was proposed that such immobilized biocatalysts could be used for therapeutic as well as biosensor applications. Soybean peroxidase immobilized on silica-coated magnetic Fe_3O_4 nanoparticles removes 99% of FA from a reaction mix compared to only 57% obtained with the free peroxidase (Silva et al., 2016). While this system was proposed for environmental remediation of effluents from wine distilleries, olive oil production and pulp and paper mills, phenolic acids cross-linked by peroxidases could also be used to generate new bioactive compounds, with higher potential than monomeric FA as antioxidants.

AnFaeA and feruloyl esterase-containing enzyme cocktails have been successfully immobilized as cross-linked enzyme aggregates (CLEAs) and used in the synthesis of alkyl hydroxycinnamates, compounds with application as antioxidants and inhibitors of LDL-oxidation (Vafiadi et al., 2008a; Vafiadi et al., 2008b). Immobilization of eight FAEs from *M. thermophila* and *T. wortmanni* using CLEA methodology demonstrated that conditions for immobilization have to be carefully designed individually for each enzyme, since the maximum activity and enzyme stability can vary in different FAE preparations (Zerva et al., 2018). Mesoporous materials have been used as enzyme immobilization supports due to their large surface area, allowing high enzyme loading, their high mechanical stability, and the ability to adjust the pore size of the material to the dimensions of the enzyme of interest.

Immobilization of FoFaeC from *F. oxysporum* on a mesoporous silica support was shown to be correlated with the pH, with a one unit shift in the optimal transesterification pH (Thörn et al., 2011; Thörn et al., 2013). Accessibility to the active site of the FAE was influenced by the surface charge distribution around the active site pocket, indicating that the esterase can adopt different orientations within the silica pores, which are pH-dependent (Thörn et al., 2013) . Other factors can influence the interaction of FAEs with their proposed

substrate, or can alter the specificity of the FAE. The physical adsorption stability of the esterase can be affected by the hydrophobicity, crystallinity and surface charge of the material it is interacting with, whether it is an immobilization support or the lignocellulosic biomass itself. The adsorption of AnFaeA onto a charged surface was simulated and shown to be regulated by electrostatic forces between the protein and the surface, in a way that such interaction can be weakened or strengthened with buffer ionic strength (Liu et al., 2015). A positively charged surface at low surface charge density and high ionic strength can maximize the control and utilization of an immobilized FAE.

The addition of organic co-solvents can expand the use of FAEs in lignocellulose deconstruction by altering the solubility of substrates or changing the solubility of lignocellulose components. A study by Faulds et al. (2011) demonstrated that low concentrations of DMSO (<20% v/v) enhanced and broadened the hydrolytic capacity of FAEs against model compounds, including acetylated compounds, possibly through an active side rearrangement. Ionic liquids (IL) can be used for both hydrolytic and synthetic reactions involving FAEs, but some FAEs have been shown to be more unstable in ILs than others (Zeuner et al., 2011). Stability in ILs was linked to being both anion dependent and enzyme structure dependent, with the more lipase-like FAEs being more stable.

The immobilization of four FAEs, MtFaeA1, MtFaeA2, MtFaeB1, and MtFaeB2 from *M. thermophila,* was studied and optimized via physical adsorption onto various mesoporous silica particles with pore diameters varying from 6.6 nm to 10.9 nm (Hüttner et al., 2017). Using crude enzyme preparations, enrichment of immobilized FAEs was observed in function on pore diameter and protein size. The immobilized enzymes were successfully used for the synthesis of butyl ferulate through transesterification of methyl ferulate with 1-butanol. Although the highest butyl ferulate yields are obtained with the free enzyme, the synthesis-to-hydrolysis ratio was higher when using immobilized enzymes. In addition, over 90% of the initial activity was observed in a reusability experiment after nine reaction cycles, each lasting 24 h. Rinsing with solvent to remove water from the immobilized enzymes further improved their activity. This study demonstrates the suitability of immobilized crude enzyme preparations in the development of biocatalysts for esterification reactions (Hüttner et al., 2017).

The immobilization of a commercially available feruloyl esterase, E-FAERU, on mesoporous silica by physical adsorption results in lower transesterification efficiency, since the hydrolysis reaction is preferred by E-FAERU, regardless of whether it is free or

immobilized (Bonzom et al., 2018). This result demonstrates that enzyme immobilization is enzyme-specific and cannot be regarded as reflecting the general behavior of FAEs.

11. Synthesis of esters-products by esterification activity

Feruloyl esters and phenolic ester sugars have important biological functions, as antitumor, antimicrobial, antiviral and anti-inflammatory agents (Paiva et al., 2013). FAEs may catalyze esterification and transesterification in non- or low-aqueous solvents to produce the esterified compounds with novel physicochemical characteristics, expanding their use in a variety of food and pharmaceutical applications (Nieter et al., 2016). Esterification can be used to modify the physical properties of FA and various hydroxycinnamic acids, with widespread industrial potential due to their antioxidant properties. For industrial applications, transesterification reactions are more expensive and complicated than esterification reactions, since FA cannot be directly used as the donor in transesterification reactions. A major obstacle for application of FA in oil-based food processing and other corresponding industries is its low solubility and stability in hydrophobic media, to overcome this limitation, it has been reported that modification of FA through esterification with aliphatic alcohols or transesterification with triacylglycerols (Vafiadi et al., 2008b). Current knowledge of biotechnological approaches for the biosynthesis of ferulate derivatives is summarized in **Fig. 6**.

There has been remarkable progress in synthesizing many feruloylated compounds to improve the water solubility of FA by linking it to hydrophilic compounds, such as glycerol and sugars. In turn, the alteration of FA via esterification with aliphatic alcohols results in lipophilic byproducts (Kikugawa et al., 2012; Kikugawa et al., 2016). Feruloyl glycerols (FGs) consist of FA esterified to glycerol, a compound naturally found in plants, which present higher solubility in water than free FA. Natural derivatives of FA, FG present many biological functions as natural UV light filters and antioxidants in the chemical, food, and drug industries (Compton et al., 2012). However, it has been reported that it is difficult to separate FGs from natural raw materials since the contents of monoferuloyl glycerol (MFG) and diferuloyl glycerol (DFG) are lower than 0.1% (Sun & Chen, 2015). In comparison to chemical methods, enzymatic synthesis of FGs involves high catalytic efficiency in mild reaction conditions, low energy consumption and high enantioselectivity (Compton et al., 2012).

An important proof-of-principle milestone was achieved with the synthesis of feruloylated L-arabinose by MtFaeC from *M. thermophila* (Topakas et al., 2005), which is the first demonstration of enzymatic feruloylation of carbohydrates. In additional studies, FAEs have been used as synthetic tools for the esterification of hydroxycinnamic acids to polyols, mono- and oligosaccharides (Tsuchiyama et al., 2006; Vafiadi et al., 2007; Vafiadi et al., 2006). Commercial enzyme preparations are able to perform transesterifications of FA to various glycoside esters, using FA esters as FA donors (Kelle et al., 2016; Tsuchiyama et al., 2006). AnFaeA is able to synthesize 1-glycerol ferulate in a mixture of 1% FA, 85% glycerol and 5% DMSO at pH 4.0, 50 °C for 30 min (Tsuchiyama et al., 2006). Kikugawa and coworkers synthesized water-soluble FA derivatives by esterification of FA with diglycerol using FAE purified from a commercial enzyme preparation produced by *A. niger* (Kikugawa et al., 2012). The major reaction product was determined to be γ-feruloyl-α,α′-diglycerol by NMR and electrospray ionization mass spectrometry analysis. Feruloyl diglycerol-1 is a sticky liquid whose water solubility (>980 mg/ml) is dramatically higher than that of FA (0.69 mg/ml).

Zeng and coworkers conducted a study using AoFaeA from *A. oryzae* expressed in *P. pastoris* to produce two feruloyl glycerol isomers, with the ester bond occurring at either the internal hydroxyl group of glycerol (2-FG) or at one of the terminal hydroxyl groups (1-FG) (Zeng et al., 2014). The maximum esterification yield reached 60.3% at water content of 20%, the major product being 1-FG and the minor product being 2-FG. Est1 from *P. sapidus* was applied for transferuloylation of different monosaccharides (D-glucose, D-fructose, D-galactose) and disaccharides (D-sucrose, D-lactose, D-maltose) using methyl ferulate as a FA donor (Kelle et al., 2016). Fortunately, there is a new development on this front. During transesterification with glucose, fructose and galactose, related substances were identified via LC-MS as the corresponding feruloylated saccharides. Five FAEs, MtFaeA1, MtFaeA2, MtFaeB1, and MtFaeB2 from *M. thermophila* C1 and MtFae1a from *M. thermophila* ATCC 42464, were tested for their ability to catalyze the transesterification of vinyl ferulate with prenol in detergentless microemulsions forming a novel feruloylated derivative, prenyl ferulate (Antonopoulou et al., 2017). The wild-type and evolved variants of MtFae1a from *M. thermophila* were able to catalyze prenyl ferulate, prenyl caffeate, glyceryl caffeate, glyceryl ferulate, n-butyl ferulate and 5-O-feruloyl-L-arabinose by transesterification using vinyl ferulate, vinyl caffeate, fatty alcohols and carbohydrates as acyl donors (Varriale et al., 2018).

12. Future perspectives

In the plant cell wall, FA is an essential component cross-linking polysaccharides to lignin, increasing the cell wall's resistance to hydrolysis. FAE employment with accessory enzymes, as xylanases and arabinofuranosidases, improves lignocellulosic biomass conversion to biofuel, helping to achieve the goals set for cost-effective production of alternative and renewable fuels and chemicals. Future investigations should center upon more complex enzymatic cocktails containing FAE, or mixtures of cocktails, that accurately reflect the complexity of plant cell walls. Thereupon, recent concepts for producing FAEs in plants, via the carefully controlled expression of CAZymes in cell walls for the purpose of initiating or even completing digestion, could well hold such promise.

Even though most studies have produced and characterized FAEs, recent developments have highlighted exceptional potential in employing the genome mining strategy to discover novel FAE encoding genes, and heterologous expression and biochemical characterization allows for the unraveling of powerful FAEs with hitherto unknown properties. With the emergence of molecular approaches to selectively alter plant cell wall structure, hydroxycinnamate-derived materials and chemicals are expected to find increasingly extensive applications, opening up new paths for the fine chemical and fuel industries.

13. Conclusion

This review presents a critical assessment comparing various strategies for FAE valorization, highlighting the recent advances in the biochemical properties, biodiversity and biotechnological applications of FAEs. Evaluation of the recent scientific literature shows that FAEs are extensively applied for obtaining FA from agricultural by-products waste and subsequent bioconversion to high added-value aromatic compounds, and as biosynthetic tools to catalyze esterification and transesterification of esters of hydroxycinnamic acids. FA and derivatives released from lignocellulose are employed as pharmaceutical agents, as food chemicals, and in the fine chemical industry.

Acknowledgments

This work was supported by grants from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível

Superior (CAPES) and Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, contract numbers 2014/18714-2 and 2017/00525-0).

Conflict of interest

The authors have declared that no competing interests exist.

References

1. Andersen, A., Svendsen, A., Vind, J., Lassen, S.F., Hjort, C., Borch, K., Patkar, S.A. 2002. Studies on ferulic acid esterase activity in fungal lipases and cutinases. *Colloids Surf. B Biointerfaces*, **26**(1), 47-55.
2. Antonopoulou, I., Iancu, L., Jutten, P., Piechot, A., Rova, U., Christakopoulos, P. 2019. Screening of novel feruloyl esterases from *Talaromyces wortmannii* for the development of efficient and sustainable syntheses of feruloyl derivatives. *Enzyme. Microb. Technol.*, **120**, 124-135.
3. Antonopoulou, I., Leonov, L., Jutten, P., Cerullo, G., Faraco, V., Papadopoulou, A., Kletsas, D., Ralli, M., Rova, U., Christakopoulos, P. 2017. Optimized synthesis of novel prenyl ferulate performed by feruloyl esterases from *Myceliophthora thermophil*a in microemulsions. *Appl. Microbiol. Biotechnol.*, **101**(8), 3213-3226.
4. Badary, O.A., Awad, A.S., Sherief, M.A., Hamada, F.M. 2006. In vitro and in vivo effects of ferulic acid on gastrointestinal motility: inhibition of cisplatin-induced delay in gastric emptying in rats. *World J. Gastroenterol.*, **12**(33), 5363-7.
5. Benoit, I., Asther, M., Sulzenbacher, G., Record, E., Marmuse, L., Parsiegla, G., Gimbert, I., Asther, M., Bignon, C. 2006a. Respective importance of protein folding and glycosylation in the thermal stability of recombinant feruloyl esterase A. *FEBS Lett.*, **580**(25), 5815-21.
6. Benoit, I., Danchin, E.G., Bleichrodt, R.J., de Vries, R.P. 2008. Biotechnological applications and potential of fungal feruloyl esterases based on prevalence, classification and biochemical diversity. *Biotechnol. Lett.*, **30**(3), 387-96.
7. Benoit, I., Navarro, D., Marnet, N., Rakotomanomana, N., Lesage-Meessen, L., Sigoillot, J.-C., Asther, M., Asther, M. 2006b. Feruloyl esterases as a tool for the release of phenolic compounds from agro-industrial by-products. *Carbohydr. Res.*, **341**(11), 1820-1827.
8. Bento-Silva, A., Patto, M.C.V., Bronze, M.R. 2018. Relevance, structure and analysis of ferulic acid in maize cell walls. *Food Chem.*, **246**, 360-378.
9. Blum, D., Kataeva, I., Li, X., Ljungdahl, L. 2000. Feruloyl esterase activity of the *Clostridium thermocellum* cellulosome can be attributed to previously unknown domains of XynY and XynZ. *J. Bacteriol.*, **182**(5), 1346-51.
10. Bonzom, C., Schild, L., Gustafsson, H., Olsson, L. 2018. Feruloyl esterase immobilization in mesoporous silica particles and characterization in hydrolysis and transesterification. *BMC Biochem.*, **19**(1), 1.
11. Borges, A., Ferreira, C., Saavedra, M.J., Simões, M. 2013. Antibacterial activity and mode of action of ferulic and gallic acids against pathogenic bacteria. *Microb. Drug Resist.*, **19**(4), 256-65.
12. Braga, C.M.P., Delabona, P.D.S., Lima, D., Paixão, D.A.A., Pradella, J., Farinas, C.S. 2014. Addition of feruloyl esterase and xylanase produced on-site improves sugarcane bagasse hydrolysis. *Bioresour. Technol.*, **170**, 316-324.
13. Cao, L.C., Chen, R., Xie, W., Liu, Y.H. 2015. Enhancing the Thermostability of Feruloyl Esterase EstF27 by Directed Evolution and the Underlying Structural Basis. *J. Agric. Food Chem.*, **63**(37), 8225-33.
14. Chandrasekharaiah, M., Thulasi, A., Vijayarani, K., Kumar, D.P., Santosh, S.S., Palanivel, C., Jose, V.L., Sampath, K.T. 2012. Expression and biochemical characterization of two novel feruloyl esterases derived from fecal samples of *Rusa unicolor* and *Equus burchelli*. *Gene*, **500**(1), 134-9.
15. Chen, J., Lin, D., Zhang, C., Li, G., Zhang, N., Ruan, L., Yan, Q., Li, J., Yu, X., Xie, X., Pang, C., Cao, L., Pan, J., Xu, Y. 2015. Antidepressant-like effects of ferulic acid: involvement of serotonergic and norepinergic systems. *Metab. Brain Dis.*, **30**(1), 129-36.
16. Compton, D.L., Laszlo, J.A., Evans, K.O. 2012. Antioxidant properties of feruloyl glycerol derivatives. *Ind. Crops Prod.*, **36**(1), 217-221.
17. Crepin, V., Faulds, C., Connerton, I. 2003a. A non-modular type B feruloyl esterase from *Neurospora crassa* exhibits concentration-dependent substrate inhibition. *Biochem. J.*, **370**(2), 417-27.

18. Crepin, V., Faulds, C., Connerton, I. 2003b. Production and characterization of the *Talaromyces stipitatus* feruloyl esterase FAEC in *Pichia pastoris*: identification of the nucleophilic serine. *Protein Expr. Purif.*, **29**(2), 176-84.
19. Crepin, V.F., Faulds, C.B., Connerton, I.F. 2004. Functional classification of the microbial feruloyl esterases. *Appl. Microbiol. Biotechnol.*, **63**(6), 647-52.
20. Damásio, A.R.L., Braga, C.M.P., Brenelli, L.B., Citadini, A.P., Mandelli, F., Cota, J., de Almeida, R.F., Salvador, V.H., Paixao, D.A.A., Segato, F., Mercadante, A.Z., Neto, M.O., dos Santos, W.D., Squina, F.M. 2013. Biomass-to-bio-products application of feruloyl esterase from *Aspergillus clavatus*. *Appl. Microbiol. Biotechnol.*, **97**(15), 6759-67.
21. de Vries, R.P., Michelsen, B., Poulsen, C., Kroon, P., van den Heuvel, R., Faulds, C., Williamson, G., van den Hombergh, J., Visser, J. 1997. The faeA genes from *Aspergillus niger* and *Aspergillus tubingensis* encode ferulic acid esterases involved in degradation of complex cell wall polysaccharides. *Appl. Environ. Microbiol.*, **63**(12), 4638–4644.
22. de Vries, R.P., vanKuyk, P.A., Kester, H.C.M., Visser, J. 2002. The *Aspergillus niger* faeB gene encodes a second feruloyl esterase involved in pectin and xylan degradation and is specifically induced in the presence of aromatic compounds. *Biochem. J.*, **363**, 377-86.
23. Debeire, P., Khoune, P., Jeltsch, J.-M., Phalip, V. 2012. Product patterns of a feruloyl esterase from *Aspergillus nidulans* on large feruloyl-arabino-xylo-oligosaccharides from wheat bran. *Bioresour. Technol.*, **119**, 425-428.
24. Dilokpimol, A., Mäkelä, M.R., Aguilar-Pontes, M.V., Benoit-Gelber, I., Hilden, K.S., de Vries, R.P. 2016. Diversity of fungal feruloyl esterases: updated phylogenetic classification, properties, and industrial applications. *Biotechnol. Biofuels*, **9**, 231.
25. Dilokpimol, A., Mäkelä, M.R., Mansouri, S., Belova, O., Waterstraat, M., Bunzel, M., de Vries, R.P., Hilden, K.S. 2017. Expanding the feruloyl esterase gene family of *Aspergillus niger* by characterization of a feruloyl esterase, FaeC. *N. Biotechnol.*, **37**(Pt B), 200-209.
26. Dilokpimol, A., Mäkelä, M.R., Varriale, S., Zhou, M., Cerullo, G., Gidijala, L., Hinkka, H., Bras, J.L.A., Jutten, P., Piechot, A., Verhaert, R., Hilden, K.S., Faraco, V., de Vries, R.P. 2018. Fungal feruloyl esterases: Functional validation of genome mining based enzyme discovery including uncharacterized subfamilies. *N. Biotechnol.*, **41**, 9-14.
27. Donaghy, J., McKay, A.M. 2003. Purification and characterization of a feruloyl esterase from the fungus *Penicillium expansum*. *J. Appl. Microbiol.*, **83**(6), 718-726.
28. dos Santos, W.D., Ferrarese, M.L.L., Ferrarese-Filho, O. 2008. Ferulic acid: an allelochemical troublemaker. *Funct. Plant Sci. Biotechnol.*, **2**, 47-55.
29. Dyk, J.S.V., Pletschke, B.I. 2012. A review of lignocellulose bioconversion using enzymatic hydrolysis and synergistic cooperation between enzymes - factors affecting enzymes, conversion and synergy. *Biotechnol. Adv.*, **30**(6), 1458-80.
30. Esteban-Torres, M., Reveron, I., Mancheno, J.M., de Las Rivas, B., Munoz, R. 2013. Characterization of a feruloyl esterase from *Lactobacillus plantarum*. *Appl. Environ. Microbiol.*, **79**(17), 5130-6.
31. Faulds, C., Williamson, G. 1991. The purification and characterization of 4-hydroxy-3-methoxycinnamic (ferulic) acid esterase from *Streptomyces olivochromogenes*. *J. Gen. Microbiol.*, **137**(10), 2339-45.
32. Faulds, C., Williamson, G. 1994. Purification and characterization of a ferulic acid esterase (FAE-III) from *Aspergillus niger*: Specificity for the phenolic moiety and binding to microcrystalline cellulose. *Microbiology*, **140**(4), 779-787.
33. Faulds, C.B., Mandalari, G., Lo Curto, R.B., Bisignano, G., Christakopoulos, P., Waldron, K. 2006. Synergy between xylanases from glycoside hydrolase family 10 and family 11 and a feruloyl esterase in the release of phenolic acids from cereal arabinoxylan. *Appl. Microbiol. Biotechnol.*, **71**(5), 622-9.
34. Faulds, C.B., Mandalari, G., LoCurto, R., Bisignano, G., Waldron, K.W. 2004. Arabinoxylan and mono- and dimeric ferulic acid release from brewer's grain and wheat bran by feruloyl esterases and glycosyl hydrolases from *Humicola insolens*. *Appl. Microbiol. Biotechnol.*, **64**(5), 644-50.
35. Faulds, C.B., Molina, R., Gonzalez, R., Husband, F., Juge, N., Sanz-Aparicio, J., Hermoso, J.A. 2005. Probing the determinants of substrate specificity of a feruloyl esterase, AnFaeA, from *Aspergillus niger*. *FEBS J.*, **272**(17), 4362-71.

36. Faulds, C.B., Perez-Boada, M., Martinez, A.T. 2011. Influence of organic co-solvents on the activity and substrate specificity of feruloyl esterases. *Bioresour. Technol*, **102**(8), 4962-7.
37. Faulds, C.B., Williamson, G. 1993. Ferulic acid esterase from *Aspergillus niger*: Purification and partial characterisation of two forms from a commercial source of pectinase. *Biotechnol. Appl. Biochem.*, **17**, 349–359.
38. Fazary, A.E., Hamad, H.A., Lee, J.C., Koskei, T., Lee, C.K., Ju, Y.H. 2010. Expression of feruloyl esterase from *Aspergillus awamori* in *Escherichia coli*: Characterization and crystal studies of the recombinant enzyme. *Int. J. Biol. Macromol.*, **46**(4), 440-4.
39. Furuya, T., Kuroiwa, M., Kino, K. 2017. Biotechnological production of vanillin using immobilized enzymes. *J. Biotechnol.*, **243**, 25-28.
40. Gao, L., Wang, M., Chen, S., Zhang, D. 2016. Biochemical characterization of a novel feruloyl esterase from *Penicillium piceum* and its application in biomass bioconversion. *J. Mol. Catal., B Enzym.*, **133**, S388-S394.
41. Garcia-Conesa, M., Crepin, V., Goldson, A., Williamson, G., Cummings, N., Connerton, I., Faulds, C., Kroon, P. 2004. The feruloyl esterase system of *Talaromyces stipitatus*: production of three discrete feruloyl esterases, including a novel enzyme, TsFaeC, with a broad substrate specificity. *J. Biotechnol.*, **108**(3), 227-41.
42. Goldstone, D.C., Villas-Boas, S.G., Till, M., Kelly, W.J., Attwood, G.T., Arcus, V.L. 2010. Structural and functional characterization of a promiscuous feruloyl esterase (Est1E) from the rumen bacterium *Butyrivibrio proteoclasticus*. *Proteins*, **78**(6), 1457-69.
43. Gong, Y.Y., Yin, X., Zhang, H.M., Wu, M.C., Tang, C.D., Wang, J.Q., Pang, Q.F. 2013. Cloning, expression of a feruloyl esterase from *Aspergillus usamii* E001 and its applicability in generating ferulic acid from wheat bran. *J. Ind. Microbiol. Biotechnol.*, **40**(12), 1433-41.
44. Gopalan, N., Rodriguez-Duran, L.V., Saucedo-Castaneda, G., Nampoothiri, K.M. 2015. Review on technological and scientific aspects of feruloyl esterases: A versatile enzyme for biorefining of biomass. *Bioresour. Technol.*, **193**, 534-44.
45. Gottschalk, L.M.F., Oliveira, R.A., Bon, E.P.S. 2010. Cellulases, xylanases, β-glucosidase and ferulic acid esterase produced by *Trichoderma* and *Aspergillus* act synergistically in the hydrolysis of sugarcane bagasse. *Biochem. Eng. J.*, **51**(1-2), 72-78.
46. Haase-Aschoff, P., Linke, D., Berger, R.G. 2013. Detection of feruloyl- and cinnamoyl esterases from basidiomycetes in the presence of interfering laccase. *Bioresour. Technol.*, **130**, 231-8.
47. Hartley, R.D., Haverkamp, J. 1984. Pyrolysis‐mass spectrometry of the phenolic constituents of plant cell walls. *J. Sci. Food Agric.*, **35**(1), 14-20.
48. Hassan, S., Hugouvieux-Cotte-Pattat, N. 2011. Identification of two feruloyl esterases in *Dickeya dadantii* 3937 and induction of the major feruloyl esterase and of pectate lyases by ferulic acid. *J. Bacteriol.*, **193**(4), 963-70.
49. Hatfield, R.D., Ralph, J. 1999. Modelling the feasibilty of intramolecular dehydro-diferulate formation in grass walls. *J. Sci. Food Agric.*, **79**, 425-427.
50. He, F., Zhang, S., Liu, X. 2015. Immobilization of feruloyl esterases on magnetic nanoparticles and its potential in production of ferulic acid. *J. Biosci. Bioeng.*, **120**(3), 330-4.
51. Hermoso, J.A., Sanz-Aparicio, J., Molina, R., Juge, N., Gonzalez, R., Faulds, C.B. 2004. The crystal structure of feruloyl esterase A from *Aspergillus niger* suggests evolutive functional convergence in feruloyl esterase family. *J. Mol. Biol.*, **338**(3), 495-506.
52. Hunt, C.J., Antonopoulou, I., Tanksale, A., Rova, U., Christakopoulos, P., Haritos, V.S. 2017. Insights into substrate binding of ferulic acid esterases by arabinose and methyl hydroxycinnamate esters and molecular docking. *Sci. Rep.*, **7**(1), 17315.
53. Hunt, C.J., Tanksale, A., Haritos, V.S. 2016. Biochemical characterization of a halotolerant feruloyl esterase from *Actinomyces* spp.: refolding and activity following thermal deactivation. *Appl. Microbiol. Biotechnol.*, **100**(4), 1777-87.
54. Hüttner, S., Gomes, M.Z.V., Iancu, L., Palmqvist, A., Olsson, L. 2017. Immobilisation on mesoporous silica and solvent rinsing improve the transesterification abilities of feruloyl esterases from *Myceliophthora thermophila*. *Bioresour. Technol.*, **239**, 57-65.
55. Jia, L., Gonçalves, G.A.L., Takasugi, Y., Mori, Y., Noda, S., Tanaka, T., Ichinose, H., Kamiya, N. 2015. Effect of pretreatment methods on the synergism of cellulase and xylanase during the hydrolysis of bagasse. *Bioresour. Technol.*, **185**, 158-164.

56. Karlen, S.D., Free, H.C., Padmakshan, D., Smith, B.G., Ralph, J., Harris, P.J. 2018. Commelinid Monocotyledon Lignins are Acylated by *p*-Coumarate. *Plant Physiol.*, **177**, 513-521.
57. Kelle, S., Nieter, A., Krings, U., Zelena, K., Linke, D., Berger, R.G. 2016. Heterologous production of a feruloyl esterase from *Pleurotus sapidus* synthesizing feruloyl-saccharide esters. *Biotechnol. Appl. Biochem.*, **63**(6), 852-862.
58. Kikugawa, M., Tsuchiyama, M., Kai, K., Sakamoto, T. 2012. Synthesis of highly water-soluble feruloyl diglycerols by esterification of an *Aspergillus niger* feruloyl esterase. *Appl. Microbiol. Biotechnol.*, **95**(3), 615-22.
59. Kikugawa, M., Tsutsuki, H., Ida, T., Nakajima, H., Ihara, H., Sakamoto, T. 2016. Water-soluble ferulic acid derivatives improve amyloid-β-induced neuronal cell death and dysmnesia through inhibition of amyloid-β aggregation. *Biosci. Biotechnol. Biochem.*, **80**(3), 547-553.
60. Kim, J.-H., Baik, S.-H. 2015. Properties of recombinant novel cinnamoyl esterase from *Lactobacillus acidophilus* F46 isolated from human intestinal bacterium. *J. Mol. Catal., B Enzym.*, **116**, 9-15.
61. Koseki, T., Hori, A., Seki, S., Murayama, T., Shiono, Y. 2009. Characterization of two distinct feruloyl esterases, AoFaeB and AoFaeC, from *Aspergillus oryzae*. *Appl. Microbiol. Biotechnol.*, **83**(4), 689-96.
62. Koseki, T., Takahashi, K., Fushinobu, S., Iefuji, H., Iwano, K., Hashizume, K., Matsuzawa, H. 2005. Mutational analysis of a feruloyl esterase from *Aspergillus awamori* involved in substrate discrimination and pH dependence. *Biochim. Biophys. Acta*, **1722**(2), 200-8.
63. Koseki, T., Takahashi, K., Handa, T., Yamane, Y., Fushinobu, S., Hashizume, K. 2006. N-linked oligosaccharides of *Aspergillus awamori* feruloyl esterase are important for thermostability and catalysis. *Biosci. Biotechnol. Biochem.*, **70**(10), 2476-80.
64. Kühnel, S., Pouvreau, L., Appeldoorn, M.M., Hinz, S.W., Schols, H.A., Gruppen, H. 2012. The ferulic acid esterases of *Chrysosporium lucknowense* C1: purification, characterization and their potential application in biorefinery. *Enzyme Microb. Technol.*, **50**(1), 77-85.
65. Levasseur, A., Benoit, I., Asther, M., Asther, M., Record, E. 2004. Homologous expression of the feruloyl esterase B gene from *Aspergillus niger* and characterization of the recombinant enzyme. *Protein Expr. Purif.*, **37**(1), 126-33.
66. Levasseur, A., Saloheimo, M., Navarro, D., Andberg, M., Monot, F., Nakari-Setala, T., Asther, M., Record, E. 2006. Production of a chimeric enzyme tool associating the *Trichoderma reesei* swollenin with the *Aspergillus niger* feruloyl esterase A for release of ferulic acid. *Appl. Microbiol. Biotechnol.*, **73**(4), 872-80.
67. Li, J., Cai, S., Luo, Y., Dong, X. 2011. Three feruloyl esterases in *Cellulosilyticum ruminicola* H1 act synergistically to hydrolyze esterified polysaccharides. *Appl. Environ. Microbiol.*, **77**(17), 6141-7.
68. Li, J., Zhou, P., Liu, H., Xiong, C., Lin, J., Xiao, W., Gong, Y., Liu, Z. 2014. Synergism of cellulase, xylanase, and pectinase on hydrolyzing sugarcane bagasse resulting from different pretreatment technologies. *Bioresour. Technol.*, **155**, 258-265.
69. Linh, T.N., Fujita, H., Sakoda, A. 2017. Release kinetics of esterified p-coumaric acid and ferulic acid from rice straw in mild alkaline solution. *Bioresour. Technol.*, **232**, 192-203.
70. Linke, D., Matthes, R., Nimtz, M., Zorn, H., Bunzel, M., Berger, R.G. 2013. An esterase from the basidiomycete *Pleurotus sapidus* hydrolyzes feruloylated saccharides. *Appl. Microbiol. Biotechnol.*, **97**(16), 7241-51.
71. Liu, J., Peng, C., Yu, G., Zhou, J. 2015. Molecular simulation study of feruloyl esterase adsorption on charged surfaces: effects of surface charge density and ionic strength. *Langmuir*, **31**(39), 10751-63.
72. Lombard, V., Golaconda Ramulu, H., Drula, E., Coutinho, P.M., Henrissat, B. 2014. The carbohydrate-active enzymes database (CAZy) in 2013. *Nucleic Acids Res*, **42**.
73. Long, L., Zhao, H., Ding, D., Xu, M., Ding, S. 2018. Heterologous expression of two *Aspergillus niger* feruloyl esterases in *Trichoderma reesei* for the production of ferulic acid from wheat bran. *Bioprocess Biosyst. Eng.*, **41**(5), 593-601.
74. Mäkelä, M.R., Dilokpimol, A., Koskela, S.M., Kuuskeri, J., de Vries, R.P., Hildén, K. 2018. Characterization of a feruloyl esterase from Aspergillus terreus facilitates the division of fungal

enzymes from Carbohydrate Esterase family 1 of the carbohydrate-active enzymes (CAZy) database. *Microbial Biotechnol.*, **11**(5), 869-880.

75. Mandalari, G., Bisignano, G., Lo Curto, R.B., Waldron, K.W., Faulds, C.B. 2008. Production of feruloyl esterases and xylanases by *Talaromyces stipitatus* and *Humicola grisea* var. thermoidea on industrial food processing by-products. *Bioresour. Technol.*, **99**(11), 5130-5133.
76. Mandelli, F., Brenelli, L.B., Almeida, R.F., Goldbeck, R., Wolf, L.D., Hoffmam, Z.B., Ruller, R., Rocha, G.J.M., Mercadante, A.Z., Squina, F.M. 2014. Simultaneous production of xylooligosaccharides and antioxidant compounds from sugarcane bagasse via enzymatic hydrolysis. *Ind. Crops Prod.*, **52**, 770-775.
77. Martinez, D., Berka, R.M., Henrissat, B., Saloheimo, M., Arvas, M., Baker, S.E., Chapman, J., Chertkov, O., Coutinho, P.M., Cullen, D., Danchin, E.G., Grigoriev, I.V., Harris, P., Jackson, M., Kubicek, C.P., Han, C.S., Ho, I., Larrondo, L.F., de Leon, A.L., Magnuson, J.K., Merino, S., Misra, M., Nelson, B., Putnam, N., Robbertse, B., Salamov, A.A., Schmoll, M., Terry, A., Thayer, N., Westerholm-Parvinen, A., Schoch, C.L., Yao, J., Barabote, R., Nelson, M.A., Detter, C., Bruce, D., Kuske, C.R., Xie, G., Richardson, P., Rokhsar, D.S., Lucas, S.M., Rubin, E.M., Dunn-Coleman, N., Ward, M., Brettin, T.S. 2008. Genome sequencing and analysis of the biomass-degrading fungus *Trichoderma reesei* (syn. *Hypocrea jecorina*). *Nat. Biotechnol.*, **26**(5), 553-60.
78. Masarin, F., Gurpilhares, D.B., Baffa, D.C.F., Barbosa, M.H.P., Carvalho, W., Ferraz, A., Milagres, A.M.F. 2011. Chemical composition and enzymatic digestibility of sugarcane clones selected for varied lignin content. *Biotechnol. Biofuels*, **4**(1), 55.
79. Mattila, P., Hellström, J., 2007. Phenolic acids in potatoes, vegetables, and some of their products. *J. Food Compost. Anal.*, **20**(3-4), 152-160.
80. McAuley, K., Svendsen, A., Patkar, S., Wilson, K. 2004. Structure of a feruloyl esterase from *Aspergillus niger*. *Acta Crystallogr. D Biol. Crystallogr.*, **D60**, 878-87.
81. McCann, M.C., Carpita, N.C. 2015. Biomass recalcitrance: a multi-scale, multi-factor, and conversion-specific property. *J Exp Bot*, **66**(14), 4109-18.
82. McClendon, S.D., Shin, H.D., Chen, R.R. 2011. Novel bacterial ferulic acid esterase from *Cellvibrio japonicus* and its application in ferulic acid release and xylan hydrolysis. *Biotechnol. Lett.*, **33**(1), 47-54.
83. Mota, T.R., Oliveira, D.M., Marchiosi, R., Ferrarese-Filho, O., dos Santos, W.D. 2018. Plant cell wall composition and enzymatic deconstruction. *AIMS Bioeng.*, **5**(1), 63-77.
84. Moukouli, M., Topakas, E., Christakopoulos, P. 2008. Cloning, characterization and functional expression of an alkalitolerant type C feruloyl esterase from *Fusarium oxysporum*. *Appl. Microbiol. Biotechnol.*, **79**(2), 245-254.
85. Nankar, R., Prabhakar, P., M, D. 2017. Hybrid drug combination: Combination of ferulic acid and metformin as anti-diabetic therapy. *Phytomedicine*, **37**, 10-13.
86. Nieter, A., Haase-Aschoff, P., Linke, D., Nimtz, M., Berger, R.G. 2014. A halotolerant type A feruloyl esterase from *Pleurotus eryngii*. *Fungal Biol.*, **118**(3), 348-57.
87. Nieter, A., Kelle, S., Linke, D., Berger, R.G. 2016. Feruloyl esterases from *Schizophyllum commune* to treat food industry side-streams. *Bioresour. Technol.*, **220**, 38-46.
88. Oleas, G., Callegari, E., Sepulveda, R., Eyzaguirre, J. 2017. Heterologous expression, purification and characterization of three novel esterases secreted by the lignocellulolytic fungus *Penicillium purpurogenum* when grown on sugar beet pulp. *Carbohydr. Res.*, **443-444**, 42-48.
89. Oliveira, D.M., Finger-Teixeira, A., Freitas, D.L., Barreto, G.E., Lima, R.B., Soares, A.R., Ferrarese-Filho, O., Marchiosi, R., dos Santos, W.D. 2017. Phenolic Compounds in Plants: Implications for Bioenergy. in: *Advances of Basic Science for Second Generation Bioethanol from Sugarcane*, (Eds.) M.S. Buckeridge, A.P. de Souza, Springer International Publishing. Cham, pp. 39-52.
90. Oliveira, D.M., Finger-Teixeira, A., Mota, T.R., Salvador, V.H., Moreira-Vilar, F.C., Molinari, H.B., Mitchell, R.A., Marchiosi, R., Ferrarese-Filho, O., dos Santos, W.D. 2015. Ferulic acid: a key component in grass lignocellulose recalcitrance to hydrolysis. *Plant Biotechnol. J.*, **13**(9), 1224-32.
91. Oliveira, D.M., Salvador, V.H., Mota, T.R., Finger-Teixeira, A., Almeida, R.F., Paixão, D.A.A., de Souza, A.P., Buckeridge, M.S., Marchiosi, R., Ferrarese-Filho, O., Squina, F.M., dos Santos,

W.D. 2016. Feruloyl esterase from *Aspergillus clavatus* improves xylan hydrolysis of sugarcane bagasse. *AIMS Bioeng.*, **4**(1), 1-11.
92. Paiva, L.B., Goldbeck, R., dos Santos, W.D., Squina, F.M. 2013. Ferulic acid and derivatives: molecules with potential application in the pharmaceutical field. *Braz. J. Pharm. Sci.* , **49**(3), 395-411.
93. Parracino, A., Gajula, G.P., di Gennaro, A.K., Neves-Petersen, M.T., Rafaelsen, J., Petersen, S.B. 2011. Towards Nanoscale Biomedical Devices in Medicine: Biofunctional and Spectroscopic Characterization of Superparamagnetic Nanoparticles. *J. Fluoresc.*, **21**(2), 663-672.
94. Perez-Boada, M., Prieto, A., Prinsen, P., Forquin-Gomez, M.P., del Rio, J.C., Gutierrez, A., Martinez, A.T., Faulds, C.B. 2014. Enzymatic degradation of Elephant grass (*Pennisetum purpureum*) stems: influence of the pith and bark in the total hydrolysis. *Bioresour. Technol.*, **167**, 469-75.
95. Ponnusamy, V.K., Nguyen, D.D., Dharmaraja, J., Shobana, S., Banu, J.R., Saratale, R.G., Chang, S.W., Kumar, G. 2019. A review on lignin structure, pretreatments, fermentation reactions and biorefinery potential. *Bioresour. Technol.*, **271**, 462-472.
96. Prates, J., Tarbouriech, N., Charnock, S., Fontes, C., Ferreira, L., Davies, G. 2001. The structure of the feruloyl esterase module of xylanase 10B from *Clostridium thermocellum* provides insights into substrate recognition. *Structure*, **9**(12), 1183-90.
97. Ramos-de-la-Peña, A.M., Contreras-Esquivel, J.C. 2016. Methods and substrates for feruloyl esterase activity detection, a review. *J. Mol. Catal., B Enzym.*, **130**, 74-87.
98. Ren, Z., Zhang, R., Li, Y., Li, Y., Yang, Z., Yang, H. 2017. Ferulic acid exerts neuroprotective effects against cerebral ischemia/reperfusion-induced injury via antioxidant and anti-apoptotic mechanisms in vitro and in vivo. *Int. J. Mol. Med.*, **40**(5), 1444-1456.
99. Romano, D., Bonomi, F., de Mattos, M.C., de Sousa Fonseca, T., de Oliveira Mda, C., Molinari, F. 2015. Esterases as stereoselective biocatalysts. *Biotechnol. Adv.*, **33**(5), 547-65.
100. Rumbold, K., Biely, P., Mastihubova, M., Gudelj, M., Gubitz, G., Robra, K.H., Prior, B.A. 2003. Purification and Properties of a Feruloyl Esterase Involved in Lignocellulose Degradation by *Aureobasidium pullulans*. *Appl. Environ. Microbiol.*, **69**(9), 5622-5626.
101. Saini, P., Gayen, P., Nayak, A., Kumar, D., Mukherjee, N., Pal, B.C., Sinha Babu, S.P. 2012. Effect of ferulic acid from *Hibiscus mutabilis* on filarial parasite *Setaria cervi*: Molecular and biochemical approaches. *Parasitol. Int.*, **61**(4), 520-531.
102. Sakamoto, T., Nishimura, S., Kato, T., Sunagawa, Y., Tsuchiyama, M., Kawasaki, H. 2005. Efficient Extraction of Ferulic Acid from Sugar Beet Pulp Using the Culture Supernatant of *Penicillium chrysogenum*. *J. Appl. Glycosci.*, **52**(2), 115-120.
103. Sang, S.L., Li, G., Hu, X.P., Liu, Y.H. 2011. Molecular Cloning, Overexpression and Characterization of a Novel Feruloyl Esterase from a Soil Metagenomic Library. *J. Mol. Microbiol. Biotechnol.*, **20**(4), 196-203.
104. Schär, A., Sprecher, I., Topakas, E., Faulds, C.B., Nystrom, L. 2016. Hydrolysis of Nonpolar n-Alkyl Ferulates by Feruloyl Esterases. *J. Agric. Food. Chem.*, **64**(45), 8549-8554.
105. Schubot, F., Kataeva, I., Blum, D., Shah, A., Ljungdahl, L., Rose, J., Wang, B. 2001. Structural basis for the substrate specificity of the feruloyl esterase domain of the cellulosomal xylanase Z from *Clostridium thermocellum*. *Biochemistry*, **40**(42), 12524-12532.
106. Schulz, K., Nieter, A., Scheu, A.K., Copa-Patino, J.L., Thiesing, D., Popper, L., Berger, R.G. 2018. A type D ferulic acid esterase from *Streptomyces werraensis* affects the volume of wheat dough pastries. *Appl. Microbiol. Biotechnol.*, **102**(3), 1269-1279.
107. Segato, F., Damasio, A.R.L., de Lucas, R.C., Squina, F.M., Prade, R.A. 2014. Genomics Review of Holocellulose Deconstruction by Aspergilli. *Microbiol. Mol. Biol. Rev.*, **78**(4), 588-613.
108. Shi, C., Zhang, X., Sun, Y., Yang, M., Song, K., Zheng, Z., Chen, Y., Liu, X., Jia, Z., Dong, R., Cui, L., Xia, X. 2016. Antimicrobial activity of ferulic acid against *Cronobacter sakazakii* and possible mechanism of action. *Foodborne Pathog. Dis.*, **13**(4).
109. Shin, H.-D., Chen, R.R. 2006. Production and characterization of a type B feruloyl esterase from *Fusarium proliferatum* NRRL 26517. *Enzyme Microb. Technol.*, **38**(3-4), 478-485.
110. Shin, H.D., Chen, R.R. 2007. A type B feruloyl esterase from *Aspergillus nidulans* with broad pH applicability. *Appl. Microbiol. Biotechnol.*, **73**(6), 1323-30.

111. Silva, E.O., Batista, R. 2017. Ferulic Acid and Naturally Occurring Compounds Bearing a Feruloyl Moiety: A Review on Their Structures, Occurrence, and Potential Health Benefits. *Compr. Rev. Food Sci. Food Saf.*, **16**(4), 580-616.
112. Silva, M.C., Torres, J.A., Nogueira, F.G.E., Tavares, T.S., Correa, A.D., Oliveira, L.C.A., Ramalho, T.C. 2016. Immobilization of soybean peroxidase on silica-coated magnetic particles: a magnetically recoverable biocatalyst for pollutant removal. *RSC Adv.*, **6**(87), 83856-83863.
113. Sompong, W., Cheng, H., Adisakwattana, S. 2017. Ferulic acid prevents methylglyoxal-induced protein glycation, DNA damage, and apoptosis in pancreatic β-cells. *J. Physiol. Biochem.* , **73**(1), 121-131.
114. Srinivasan, M., Sudheer, A.R., Menon, V.P. 2007. Ferulic Acid: Therapeutic Potential Through Its Antioxidant Property. *J. Clin. Biochem. Nutr.*, **40**, 92-100.
115. Sun, S., Chen, X. 2015. Kinetics of enzymatic synthesis of monoferuloyl glycerol and diferuloyl glycerol by transesterification in [BMIM]PF6. *Biochem. Eng. J.*, **97**, 25-31.
116. Suzuki, K., Hori, A., Kawamoto, K., Thangudu, R., Ishida, T., Igarashi, K., Samejima, M., Yamada, C., Arakawa, T., Wakagi, T., Koseki, T., Fushinobu, S. 2014. Crystal structure of a feruloyl esterase belonging to the tannase family: a disulfide bond near a catalytic triad. *Proteins*, **82**(10), 2857-67.
117. Szwajgier, D., Waśko, A., Targoński, Z., Niedźwiadek, M., Bancarzewska, M. 2010. The Use of a Novel Ferulic Acid Esterase from *Lactobacillus acidophilus* K1 for the Release of Phenolic Acids from Brewer's Spent Grain. *J. I. Brewing*, **116**(3), 293-303.
118. Terrett, O.M., Dupree, P. 2019. Covalent interactions between lignin and hemicelluloses in plant secondary cell walls. *Curr. Opin. Biotechnol.*, **56**, 97-104.
119. Thörn, C., Gustafsson, H., Olsson, L. 2011. Immobilization of feruloyl esterases in mesoporous materials leads to improved transesterification yield. *J. Mol. Catal., B Enzym.*, **72**(1), 57-64.
120. Thörn, C., Udatha, D.B.R.K.G., Zhou, H., Christakopoulos, P., Topakas, E., Olsson, L. 2013. Understanding the pH-dependent immobilization efficacy of feruloyl esterase-C on mesoporous silica and its structure–activity changes. *J. Mol. Catal., B Enzym.*, **93**, 65-72.
121. Topakas, E., Moukouli, M., Dimarogona, M., Christakopoulos, P. 2012a. Expression, characterization and structural modelling of a feruloyl esterase from the thermophilic fungus Myceliophthora thermophila. *Appl. Microbiol. Biotechnol.*, **94**, 399.
122. Topakas, E., Moukouli, M., Dimarogona, M., Christakopoulos, P. 2012b. Expression, characterization and structural modelling of a feruloyl esterase from the thermophilic fungus *Myceliophthora thermophila*. *Appl. Microbiol. Biotechnol.*, **94**(2), 399-411.
123. Topakas, E., Stamatis, H., Biely, P., Christakopoulos, P. 2004. Purification and characterization of a type B feruloyl esterase (StFAE-A) from the thermophilic fungus *Sporotrichum thermophile*. *Appl. Microbiol. Biotechnol.*, **63**, 686-690.
124. Topakas, E., Stamatis, H., Biely, P., Kekos, D., Macris, B.J., Christakopoulos, P. 2003. Purification and characterization of a feruloyl esterase from *Fusarium oxysporum* catalyzing esterification of phenolic acids in ternary water–organic solvent mixtures. *J. Biotechnol.*, **102**(1), 33-44.
125. Topakas, E., Vafiadi, C., Stamatis, H., Christakopoulos, P. 2005. *Sporotrichum thermophile* type C feruloyl esterase (StFaeC): purification, characterization, and its use for phenolic acid (sugar) ester synthesis. *Enzyme Microb. Technol.*, **36**(5), 729-736.
126. Tsuchiyama, M., Sakamoto, T., Fujita, T., Murata, S., Kawasaki, H. 2006. Esterification of ferulic acid with polyols using a ferulic acid esterase from *Aspergillus niger*. *Biochim. Biophys. Acta, Gen. Subj.*, **1760**(7), 1071-1079.
127. Udatha, D.B., Kouskoumvekaki, I., Olsson, L., Panagiotou, G. 2011. The interplay of descriptor-based computational analysis with pharmacophore modeling builds the basis for a novel classification scheme for feruloyl esterases. *Biotechnol. Adv.*, **29**(1), 94-110.
128. Udatha, D.B.R.K.G., Mapelli, V., Panagiotou, G., Olsson, L. 2012. Common and Distant Structural Characteristics of Feruloyl Esterase Families from *Aspergillus oryzae*. *PLoS One*, **7**(6), e39473.
129. Uraji, M., Arima, J., Inoue, Y., Harazono, K., Hatanaka, T. 2014. Application of Two Newly Identified and Characterized Feruloyl Esterases from *Streptomyces* sp. in the Enzymatic Production of Ferulic Acid from Agricultural Biomass. *PLoS One*, **9**(8), e104584.

130. Uraji, M., Tamura, H., Mizohata, E., Arima, J., Wan, K., Ogawa, K., Inoue, T., Hatanaka, T. 2018. Loop of *Streptomyces* Feruloyl Esterase Plays an Important Role in the Enzyme's Catalyzing the Release of Ferulic Acid from Biomass. *Appl. Environ. Microbiol.*, **84**(3).
131. Vafiadi, C., Topakas, E., Alderwick, L.J., Besra, G.S., Christakopoulos, P. 2007. Chemoenzymatic synthesis of feruloyl d-arabinose as a potential anti-mycobacterial agent. *Biotechnol. Lett.*, **29**(11), 1771-1774.
132. Vafiadi, C., Topakas, E., Alissandratos, A., Faulds, C.B., Christakopoulos, P. 2008a. Enzymatic synthesis of butyl hydroxycinnamates and their inhibitory effects on LDL-oxidation. *J. Biotechnol.*, **133**(4), 497-504.
133. Vafiadi, C., Topakas, E., Christakopoulos, P. 2008b. Preparation of multipurpose cross-linked enzyme aggregates and their application to production of alkyl ferulates. *J. Mol. Catal., B Enzym.*, **54**(1), 35-41.
134. Vafiadi, C., Topakas, E., Christakopoulos, P. 2006. Regioselective esterase-catalyzed feruloylation of L-arabinobiose. *Carbohydr. Res.*, **341**(12), 1992-1997.
135. Varriale, S., Cerullo, G., Antonopoulou, I., Christakopoulos, P., Rova, U., Tron, T., Faure, R., Jutten, P., Piechot, A., Bras, J.L.A., Fontes, C., Faraco, V. 2018. Evolution of the feruloyl esterase MtFae1a from *Myceliophthora thermophila* towards improved catalysts for antioxidants synthesis. *Appl. Microbiol. Biotechnol.*, **102**(12), 5185-5196.
136. Waldron , K.W., Ng, A., Parker Mary, L., Adrian, J.P. 1999. Ferulic Acid Dehydrodimers in the Cell Walls of Beta vulgaris and their Possible Role in Texture. *J. Sci. Food Agric.*, **74**(2), 221-228.
137. Wang, L., Li, Z., Zhu, M., Meng, L., Wang, H., Ng, T.B. 2016. An acidic feruloyl esterase from the mushroom *Lactarius hatsudake:* A potential animal feed supplement. *Int. J. Biol. Macromol.*, **93**(Pt A), 290-295.
138. Wang, L., Ma, Z., Du, F., Wang, H., Ng, T.B. 2014. Feruloyl esterase from the edible mushroom *Panus giganteus*: a potential dietary supplement. *J. Agric. Food Chem.*, **62**(31), 7822-7.
139. Wong, D.W. 2006. Feruloyl esterase: a key enzyme in biomass degradation. *Appl. Biochem. Biotechnol.*, **133**(2), 87-112.
140. Wong, D.W., Chan, V.J., Liao, H., Zidwick, M.J. 2013. Cloning of a novel feruloyl esterase gene from rumen microbial metagenome and enzyme characterization in synergism with endoxylanases. *J. Ind. Microbiol. Biotechnol.*, **40**(3-4), 287-95.
141. Wu, H., Li, H., Xue, Y., Luo, G., Gan, L., Liu, J., Mao, L., Long, M. 2017. High efficiency co-production of ferulic acid and xylooligosaccharides from wheat bran by recombinant xylanase and feruloyl esterase. *Biochem. Eng. J.*, **120**, 41-48.
142. Wu, M., Abokitse, K., Grosse, S., Leisch, H., Lau, P.C. 2012. New feruloyl esterases to access phenolic acids from grass biomass. *Appl. Biochem. Biotechnol.*, **168**(1), 129-43.
143. Xu, Z., He, H., Zhang, S., Guo, T., Kong, J. 2017. Characterization of Feruloyl Esterases Produced by the Four Lactobacillus Species: *L. amylovorus, L. acidophilus, L. farciminis* and *L. fermentum*, Isolated from Ensiled Corn Stover. *Front. Microbiol.*, **8**, 941.
144. Xue, Y., Wang, X., Chen, X., Hu, J., Gao, M.-T., Li, J. 2017. Effects of different cellulases on the release of phenolic acids from rice straw during saccharification. *Bioresour. Technol.*, **234**, 208-216.
145. Yang, S.-Q., Tang, L., Yan, Q.-J., Zhou, P., Xu, H.-B., Jiang, Z.-Q., Zhang, P. 2013. Biochemical characteristics and gene cloning of a novel thermostable feruloyl esterase from *Chaetomium* sp. *J. Mol. Catal., B Enzym.*, **97**, 328-336.
146. Yao, J., Chen, Q.L., Shen, A.X., Cao, W., Liu, Y.H. 2013. A novel feruloyl esterase from a soil metagenomic library with tannase activity. *J. Mol. Catal., B Enzym.*, **95**, 55-61.
147. Yin, X., Hu, D., Li, J.F., He, Y., Zhu, T.D., Wu, M.C. 2015a. Contribution of Disulfide Bridges to the Thermostability of a Type A Feruloyl Esterase from *Aspergillus usamii*. *PLoS One*, **10**(5), e0126864.
148. Yin, X., Li, J.F., Wang, C.J., Hu, D., Wu, Q., Gu, Y., Wu, M.C. 2015b. Improvement in the thermostability of a type A feruloyl esterase, AuFaeA, from *Aspergillus usamii* by iterative saturation mutagenesis. *Appl. Microbiol. Biotechnol.*, **99**(23), 10047-56.

149. Zeng, Y., Yin, X., Wu, M.-C., Yu, T., Feng, F., Zhu, T.-D., Pang, Q.-F. 2014. Expression of a novel feruloyl esterase from *Aspergillus oryzae* in *Pichia pastoris* with esterification activity. *J. Mol. Catal., B Enzym.*, **110**, 140-146.
150. Zeuner, B., Stahlberg, T., van Buu, O.N., Kunov-Kruse, A.J., Riisager, A., Meyer, A.S. 2011. Dependency of the hydrogen bonding capacity of the solvent anion on the thermal stability of feruloyl esterases in ionic liquid systems. *Green Chem.*, **13**(6), 1550-1557.
151. Zhang, S.-B., Pei, X.-Q., Wu, Z.-L. 2012. Multiple amino acid substitutions significantly improve the thermostability of feruloyl esterase A from *Aspergillus niger*. *Bioresour. Technol.*, **117**, 140-147.
152. Zhang, S.-B., Wu, Z.-L. 2011. Identification of amino acid residues responsible for increased thermostability of feruloyl esterase A from *Aspergillus niger* using the PoPMuSiC algorithm. *Bioresour. Technol.*, **102**(2), 2093-2096.
153. Zhang, S.B., Wang, L., Liu, Y., Zhai, H.C., Cai, J.P., Hu, Y.S. 2015. Expression of feruloyl esterase A from *Aspergillus terreus* and its application in biomass degradation. *Protein Expr. Purif.*, **115**, 153-7.
154. Zhang, S.B., Zhai, H.C., Wang, L., Yu, G.H. 2013. Expression, purification and characterization of a feruloyl esterase A from *Aspergillus flavus*. *Protein Expr. Purif.*, **92**(1), 36-40.
155. Zhao, Z., Moghadasian, M.H. 2008. Chemistry, natural sources, dietary intake and pharmacokinetic properties of ferulic acid: A review. *Food Chem.*, **109**(4), 691-702.

Figure captions

Figure 1. Chemical structures of hydroxycinnamic acids (A) and hydroxycinnamate methyl esters (B).

Figure 2. Schematic representation of arabinoxylan ester-linked with FA and FA dehydrodimers. i) FA esterified to arabinosyl residue and the site of action of FAE, ii) 8-5' diFA benzofuran form, iii) 8-8' diFA, iv) 8-8' diFA aryltetralin form, v) 8-O-4' diFA, vi) 5-5' diFA, and vii) 8-5' diFA.

Figure 3. Histogram and normal distribution of biochemical properties of microbial FAEs. Frequencies of molecular masses (kDa for monomers) (A), optimum temperature (B), optimum pH (C), and isoelectric point (D). Outlier values presented in Table 2 were excluded of analysis. n is the number of characterized enzymes used for the calculations.

Figure 4. Crystallographic structure of AnFaeA from *Aspergillus niger* (PDB entry 1UZA) (A to C) and a lipase from *Thermomyces lanuginosa* (PDB entry 1EIN) (D to E). The catalytic cleft with the substrate from both AnFaeA and lipase, which is in the open configuration (B and E). Cleft depth from AnFaeA and lipase (C and F).

Figure 5. Structure of *Aspergillus oryzae* AoFaeB (PDB entry 3WMT). The active site with Ser203, His457 and Asp417 triad (dashed circle) and the calcium ion at the lid position (solid circle) (A). Monomer of AoFaeB, showing the lid and catalytic cleft position (B), cartoon representation (C) and molecular surface (D).

Figure 6. Biotechnological approaches for FAE production and applications affording feruloylated derivatives.

Table 1. Amount of ferulic acid in different biomass sources.

Group	Species, family	Organ or tissue	Concentration of FA (mg/g)	Reference
Commelinid monocots	Maize (*Zea mays*), Poaceae	Grain	20.66	(Hartley and Haverkamp, 1984)
		Bran	26.1 to 33.0	(Zhao and Moghadasian, 2008)
		Stem	6.27	(Hartley and Haverkamp, 1984)
		Leaf blade	3.86	(Hartley and Haverkamp, 1984)
	Perennial ryegrass (*Lollium perenne*), Poaceae	Shoot	6.03	(Hartley and Haverkamp, 1984)
	Sugarcane (*Saccharum officinarum*), Poaceae	Culm bagasse	8.0 to 17.0	(Masarin et al., 2011)
	Barley (*Hordeum vulgare*), Poaceae	Stem	3.51	(Hartley and Haverkamp, 1984)
	Rice (*Oryza sativa*), Poaceae	Grain	0.091 to 0.143	(Zhao and Moghadasian, 2008)
	Nile grass (*Cyperus papyrus*), Cyperaceae	Stem	3.18	(Karlen et al., 2018)
	Ginger lily (*Hedychium gardnerianum*), Zingiberaceae	Stem	3.43	(Karlen et al., 2018)
	Pineapple (*Ananas comosus*), Bromeliaceae	Fruit core	6.84	(Karlen et al., 2018)
	Tassel cord Rush (*Baloskion tetraphyllum*), Restionaceae	Stem	2.27	(Karlen et al., 2018)
	Blue ginger (*Dichorisandra thyrsiflora*), Commelinaceae	Stem	1.07	(Karlen et al., 2018)
	Canary island date palm (*Phoenix canariensis*), Arecaceae	Leaf	0.17	(Karlen et al., 2018)
Non-commelinid plants	Onion (*Allium cepa*), Alliaceae	Bulb	0.007	(Zhao and Moghadasian, 2008)
	Beetroot (*Beta vulgaris*), Amaranthaceae	Root	6.93	(Waldron et al., 1999)
	Sugarbeet (*Beta vulgaris*), Amaranthaceae	Root	4.59	(Waldron et al., 1999)
	Spinach (*Spinacia oleraceae*), Amaranthaceae	–	0.074	(Mattila and Hellström, 2007)
	Soybean (*Glycine max*), Fabaceae	–	0.12	(Mattila and Hellström, 2007)
	White cabbage (*Brassica oleraceae*), Brassicaceae	–	0.0027	(Mattila and Hellström, 2007)
	Broccoli (*Brassica oleraceae*), Brassicaceae	–	0.041	(Mattila and Hellström, 2007)
	Tomato (*Lycopersicum esculentum*), Solanaceae	–	0.0029	(Mattila and Hellström, 2007)

–, not defined or not mentioned.

Table 1. Biochemical properties and kinect values of microbial FAEs.

Microorganism	Enzyme	MW (kDa)	p*I*	pH opt	*T* opt (°C)	*T* sta (°C)	K_m (mM)	k_{cat} (s^{-1})	k_{cat}/K_m (s^{-1} mM^{-1})	Substrate
Actynomyces sp.	ActOFaeI	32	–	6.5	30	40	2.79	278.18	99.61	MCA
Aspergillus awamori	AwFaeA	35	4.2	5.5	55	50	1.38	–	–	MFA
Aspergillus clavatus	AcFae	28.4	–	7.0	30	–	–	–	–	–
Aspergillus flavus	AfFaeA	40	–	6.0	58	–	0.44	2.21	5.02	MFA
							0.31	0.27	0.88	MpCA
							0.26	0.90	3.47	MSA
							–	–	–	MCA
Aspergillus nidulans	AN1772.2	130	4.6	7.0	45	45	0.248	–	–	MFA
							0.659	–	–	MpCA
Aspergillus niger	Fae-I	63	3.0	–	–	–	1.21	–	–	MFA
							0.15			MpCA
	Fae-II	29	3.6	–	–	–	1.11	–	–	MFA
							0.47			MpCA
	AnFaeA	36-31	3.3	5.0	55-60	–	2.08	70.74	91.0	MFA
							1.44	84.95	3.53	MSA
	AnFaeB	75	4.9	6.0	50	50	–	–	–	–
	AnFaeC	30	4.8	7.0	50	45	–	–	–	–
Aspergillus oryzae	AoFaeA	37	4.9	5.0	50	50	0.81	–	–	MFA
	AoFaeB	61	–	6.0	55	55	0.14	–	–	MFA
							0.022	–	–	MpCA
							0.104	–	–	MCA
	AoFaeC	75	–	6.0	55	60	0.10	–	–	MFA
							0.058	–	–	MpCA
							0.091	–	–	MCA
Aspergillus terreus	AtFaeA	35	–	5.0	50	–	0.61	2.59	4.25	MFA
							0.38	0.31	0.81	MpCA
							0.29	4.68	16.14	MSA
							ND	ND	ND	MCA
	AtFaeD	43	4.34	7.0	50	37	–	–	–	–
	AtFAE-1	76	–	5.0	50	55	0.08	1.32	16.58	MpCA
	AtFAE-2	23	–	5.0	40	55	0.07	1.27	18.14	MpCA
	AtFAE-3	36	–	5.0	40	55	0.07	1.32	18.89	MpCA
Aspergillus tubingensis	AtFaeA	36	3.3	5.0	60	–	–	–	–	–
Aspergillus usamii	AuFaeA	36	4.3	5.0	45	45	4.64	–	–	MFA
Aureobasidium pullulans	ApFae	210	6.5	6.7	60	60	0.050	15.3	304.78	MFA
							0.010	31.1	2,933.96	MpCA
							0.137	23.7	172.99	MSA
							0.098	30.0	306.12	MCA
Auricularia auricula-judae	EstBC	36	3.2	6.5	61-66	–	0.04	1.4	38	MFA
Butyrivibrio proteoclasticus	Est1E	31.6[1]	–	–	–	–	0.19	23.0	121.05	pNA
							0.24	24.0	100.0	pNB
Cellvibrio japonicus	CjFae1B	61	–	6.5	35-40	40	–	–	–	–
Cellulosilyticum ruminicola H1	FaeI	58	5.59	6.0-7.0	40	50	4.78	8.46	1.77	MFA
							2.50	2.34	0.94	MCA
							13.76	7.78	0.57	MpCA
	FaeII	31.5	6.02	8.0	35	45	0.36	645.9	1,794.2	MFA
							0.16	232.7	1,454.4	MCA

							0.51	310.5	608.8	MpCA
	FaeIII	42	4.98	9.0	40	50	0.03	2,130.9	71.029	MFA
							1.06	330.9	312.1	MCA
							1.26	538. 6	427.4	MpCA
Chaetomium sp. CQ31	Fae	29.6	–	7.5	60	50	0.98	–	–	MFA
Chrysosporium lucknowense	Fae1	29	5.5	6.5	45	–	–	–	–	–
	Fae2	36	5.2	7.5	40	–	–	–	–	–
	FaeB2	33	6.0	8.0	65	–	–	–	–	–
Clostridium thermocellum	XynZ	45	5.8	6.0	60	70	5.0	–	–	FAXXX
Erwinia chrysanthemi	FaeD	35	–	7.5	–	–	–	–	–	–
	FaeT	35	–	7.5	–	–	–	–	–	–
Fusarium oxysporum	FoFaeA/FaeII	27	9.9	7.0	45	45	0.58	0.65	1.13	MFA
							0.68	0.21	0.31	MpCA
							0.29	1.94	6.70	MSA
							0.81	0.13	0.16	MCA
	FoFaeB/FaeI	31	9.5	7.0	55	30	0.60	6.85	11.41	MFA
							0.20	19.88	99.44	MpCA
							1.12	0.46	0.416	MSA
							0.26	5.91	22.75	MCA
	FoFaeC	62	–	6.0	65	45	0.12	120.4	1,003.47	MFA
							0.21	222.9	1,061.43	MpCA
							0.50	8.2	16.4	MSA
							0.16	140.6	879.06	MCA
Fusarium proliferatum	FpFae	31	–	6.5–7.5	50	50	0.146	–	–	MFA
							0.263	–	–	MpCA
							0.196	–	–	MCA
Lactarius hatsudake	LhFae	55	–	4.0	30	–	0.54	–	–	MFA
Lactobacillus acidophilus	FaeLac	29-36	5.6	6.5-7.5	–	–	–	–	–	–
Lactobacillus amylovorus	FaeLam	29	–	8.0	45-50	50	–	–	–	–
Lactobacillus farciminis	FaeLfa	29	–	7.0	45-50	50	–	–	–	–
Lactobacillus fermentum	FaeLfe	29	–	6.5-7.0	37-50	50	–	–	–	–
Lactobacillus plantarum	Lp_0796	28	–	7.0	–	30	–	–	–	–
Myceliophthora thermophila	MtFaeB	33	3.1	6.0	55-60	50	0.71	3.30	4.64	MFA
							0.09	5.47	60.77	MpCA
							0.21	4.56	21.74	MCA
	MtFaeC	23	3.5	6.0	55	60	1.64	2.60	1.58	MFA
							0.59	0.53	0.90	MpCA
							0.57	0.40	0.70	MSA
							0.14	0.13	0.95	MCA
Neurospora crassa	NcFae-I	35	8.26	6.0	55	–	0.25	5.24	21	MFA
							0.021	12.19	580	MpCA
							0.02	4.8	100	MCA
Panus giganteus	PgFae	61	–	4.0	40	40	0.36	–	–	–
Penicillium expansum	PeFae	65	–	5.6	37	–	2.6	–	–	MFA
Penicillium piceum	PpFae	56	–	3.0	70	60	–	–	–	–
Pleurotus eryngii	PeFaeA	67	5.2	5.0	50	40	0.145	0.85	5.85	MFA
							0.44	3.41	7.75	FAX
Pleurotus sapidus	PsEst1	55	5.7	6.0	50	–	1.95	–	11.2	MFA
Schizophyllum commune	ScFaeD1	63	–	7.5	45	45	0.159	51.0	319.2	MFA
							0.151	105.1	696.3	MpCA
							0.123	38.4	310.6	MCA
	ScFaeD2	54	–	7.5	45	45	0.146	54.7	372.6	MFA
							0.137	91.4	666.2	MpCA
							0.123	34.1	275.1	MCA
Sorangium cellulosum	ScFae1	35	5.07	7.0	–	45	0.74	8.3	11.21	MFA
							0.43	9.6	22.32	MSA
	ScFae2	34	4.67	6.0-8.0	–	55	0.22	8.2	37.27	MFA
							0.18	6.5	36.11	MSA

Streptomyces sp.	R18	38	–	7.5	50	45	4.99	–	–	MFA
							4.31	–	–	MpCA
							3.31	–	–	MCA
							9.39	–	–	MSA
	R43	52	–	7.0	40	40	4.41	–	–	MFA
							3.00	–	–	MpCA
							2.61	–	–	MCA
							0.54	–	–	MSA
Streptomyces olivochromogenes	SoFae	29	7.9	5.5	30	-	1.86	–	–	MFA
Streptomyces werraensis	SwFaeD	48	–	7.5	40	45	0.23	1.98	8.73	MFA
							0.12	0.89	7.67	MSA
Talaromyces stipitatus	TsFaeA	35	3.5	–	–	–	–	–	–	–
	TsFaeB	35	5.3	–	–	–	–	–	–	–
	TsFaeC	65	4.6	6.0-7.0	60		0.04	9.65	323.0	MFA
							0.01	9.55	746.0	MpCA
							0.37	9.26	25.0	MSA
Talaromyces wortmanni	Fae125	40	5.6	–	–	–	–	–	–	–
	Fae7262	43	5.2	–	–	–	–	–	–	–
	Fae68	58.8	4.9	–	–	–	–	–	–	–
[2] Cotton soil metagenomics	Tan410	55	4.7	7.0	35	40	–	–	–	–
[2] Fecal samples of *Rusa unicolor* and *Equus burchelli*	Tvms10	18.6	–	7.0	35	40	–	–	–	–
	Tvmz2a	31.2	–	8.0	38	40	–	–	–	–
[2] Microflora of a cow's rumen	RuFae2	29	8.5	7.0	50	50	–	–	–	–

[1]Dimeric protein. [2]Metagenomic analysis. FAXX: *O*-[5-*O*-(transferuloyl)-α-L-arabinofuranosyl]-(1→3)-*O*-β-D-xylopyranosyl-(1→4)-D-xylopyranose; FAXXX: *O*-β-D-xylopyranosyl-(1→4)-*O*-[5-*O*-(trans-feruloyl)-α-L-arabinofuranosyl-(1→3)]-*O*-β-D-xylopyranosyl-(1→4)-D-xylopyranose; MCA: methyl caffeate; MFA: methyl ferulate; MpCA: methyl *p*-coumarate; MSA: methyl sinapate; ND, not detected; pNA: *p*-Nitrophenyl acetate; pNB: *p*-Nitrophenyl butyrate; pNF: *p*-Nitrophenyl ferulate; –, not detected or not mentioned.

Table 2. Comparison in protein glycosylation effects of heterologous expressed FAEs.

Organism	Protein	Expressed host	MW (kDa)		Glycosylation site		Optimum temperature (°C	
			Glycosylated	Non-glycosylated	O	N	Glycosylated	Non-glycosyl
Aureobasidium pullulans	ApFae	-	210	110	–	–	60	–
Aspergillus nidulans	AN1772.2	*S. cerevisiae*	130	56	–	6	45	–
Aspergillus flavus	AfFaeA	*P. pastoris*	40	28.164[#]	–	2	58	–
Aspergillus niger	AnFaeA	*A. niger*[1] *and E. coli*[2]	30.246	28.552	–	1	55	50
Aspergillus niger	AnFaeB	*A. niger CMICC 298302*	74	60	–	18	–	–
Aspergillus niger N402	AnFaeC	*P. pastoris*	30	30	–	–	50	–
Aspergillus niger	AnFaeD	*P. pastoris*	43	–	2	7	–	–
Aspergillus oryzae	AoFaeB	*P. pastoris*	61	55	–	13	-	–
Aspergillus oryzae	AoFaeC	*P. pastoris*	75	55	–	10	-	–
Aspergillus terreus	AtFaeA	*P. pastoris*	40 and 37	35	53	2	50	–
Myceliophthora thermophila	MtFae1	*P. pastoris*	39	32.303[#]	–	3	50	–
Neurospora crassa	NcFae-I	*P. pastoris*	35.04	29.286[#]	–	4	55	–
Penicillium purpurogenum	PpFaeA	*P. pastoris*	35	32	–	–	48	–
Pleurotus sapidus	PsEst1	*P. pastoris*	70	60	1	3	50	–
Talaromyces stipitatus	TsFaeC	*P. pastoris*	66	55.340[#]	–	–	60	–
Talaromyces wortmannii	Fae68	*M. thermophila*	58.8	–	11	10	–	–
	Fae125	*C1*	40	–	22	0	–	–
	Fae7262		43	–	23	2	–	–

[1] Glycosylated. [2] Non-glycosylated. [#] Predicted molecular mass. * Tm (melting temperature).
–, not detected or not mentioned.

HIGHLIGHTS (maximum 85 characters)

- Critical assessment comparing various strategies for feruloyl esterase valorization
- Feruloyl esterases are versatile biocatalysts for the production of hydroxycinnamates
- The potential of feruloyl esterases for biotechnological applications is highlighted
- Biosynthetic tools for esterification and transesterification of bioactive compounds