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Methods and substrates for feruloyl esterase activity detection, a review

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ABSTRACT

Ferulic acid esterases (FAEs) are enzymes with high potential in biochemistry and biotechnology due to its role in the hydroxycinnamic acids liberation from agro industrial waste materials. These enzymes play a key role in the non woody plants delignification, in the saccharification processes, in the increase of waste products digestibility and in the esterification of hydroxycinnamic acids. Because of this, different industries have focused their attention on FAEs to obtain products such as human and animal food, pulp and paper, fuel ethanol and pharmaceutical preparations. The growing importance of FAEs in the industry requires fast, simple and sustainable analytical methods. The aim of this mini review is to provide an updated revision of the available assays and substrates to analyze FAE activity. Qualitative and quantitative methods for the enzyme, substrates and specificity are considered in this compilation.

Contents

1. Introduction

Feruloyl esterase enzymes (E.C. 3.1.1.73) are also known as ferulic acid esterases (FAE), cinnamoyl esterases and cinnamoyl ester hydrolases [1]. FAE enzymes represent a subclass of the carboxylic ester hydrolases, which cleave the ester linkages between hydroxycinnamic acids in the plant cell walls and sugars [2]. FAEs liberate phenolic acids such as ferulic acid and *p*-coumaric acid, and their dimers from naturally occurring hemicelluloses and pectins, where they mainly occur as esters with L-arabinofuranose containing polysaccharides such as L-arabino-D-xylans and L-arabinans [3].

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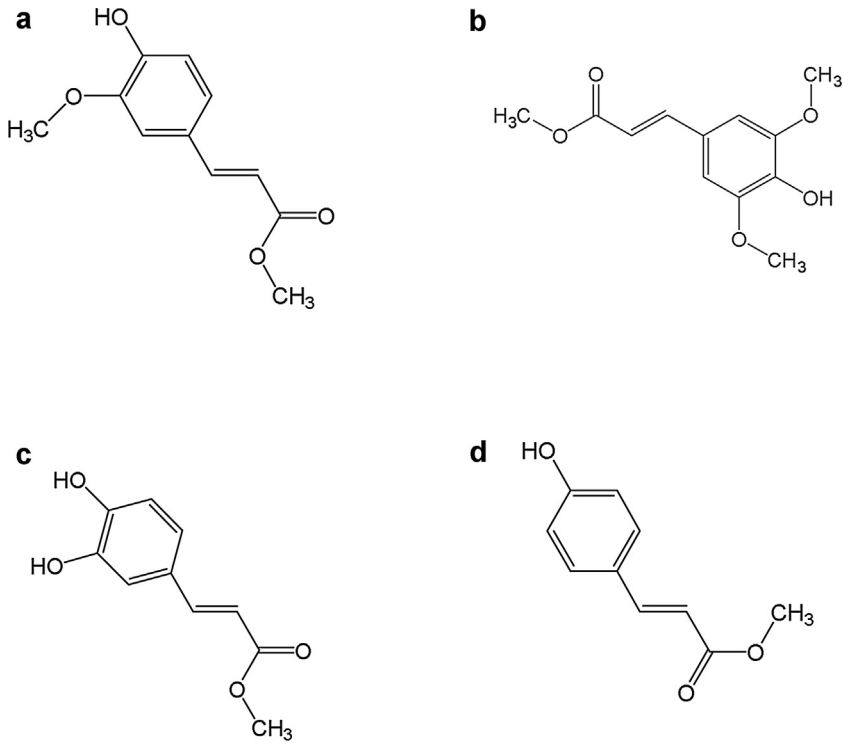

Fig. 1. Synthetic substrates used for FAE classification, (a) methyl ferulate, (b) methyl sinapate, (c) methyl caffeate and d. methyl *p*-coumarate.

Based on the specificity against synthetic substrates (Fig. 1) such as methyl ferulate (a) (MFA), methyl sinapate (b) (MSA), methyl caffeate (c) (MCA) and methyl *p*-coumarate (d) (MpCA), FAEs have been sub-classified into four types: A, B, C and D [4]. Type A shows preference for the phenolic moiety of the substrate containing methoxy substitutions, while type B shows complementary activity to type A, showing preference for substrates containing one or two hydroxyl substitutions. Type C and D show a broad specificity against synthetic hydroxycinnamic acids showing difference only in the ability to liberate 5-5′ diferulic acid [3].

Several FAEs have been studied and they often have different substrate specificities and physical properties. Most FAEs have been isolated from fungal (*Aspergillus* spp., *Penicillium funicolusum*, *Talaromyces stipitatus* (*T. stipitatus*)) rather than from bacterial sources (*Clostridium* spp., *Pseudomonas fluorescens*) [3,5–7]. Moreover, FAE activity also has been reported being endogenous to plants in germinating barley, being involved in the cell wall extension process [8].

The food industry has focused attention on the hydroxycinnamic acids because they are widely distributed in plants, fruits and vegetables and because their antioxidant properties [9]. As consequence, agro industrial by-products represent a source of added value phenolic acids after being treated with FAEs. Food materials have been synthesized by using these enzymes, for instance the enzymatic feruloylated saccharides [10]. The industry of fuel ethanol (bio ethanol) also is interested because their role in the bio conversion of lignocellulosic wastes to fermentable sugars [11]. Moreover, the health and pharmaceutical industries have used FAEs to synthesize novel bioactive compounds [12]. In addition, the potential use of FAEs has been considered for the biocatalytic conversion of ferulic acid into styrene, polymers, epoxides alkyl benzenes, vanillic acid derivatives, protocatechuic acid related catechols, guaiacol, catechol and vanillin [13]. FAEs also have application in the pulp and paper industry, increasing the amount of phenolic compounds liberated, and de structuring hemicellulose and lignin, improving the soda cooking conditions of pulps, reducing the amount of chemicals required for the papermaking process [14]. The farming industry also is interested because the pretreatment of wheat, maize and rice bran with FAEs can increase the digestibility and the calorific value [15].

Several methods have been developed for the FAE activity determination and natural and synthetic substrates have been used for this purpose. Despite of this, a review of the available methodologies developed for this enzyme activity does not exist. Because of this reason, the aim of this mini review is to provide and updated revision of the most important qualitative and quantitative methods for the FAE activity analysis and quantification, including the natural and synthetic substrates and specificity against different substrates.

2. Quantitative and qualitative methods for feruloyl esterase detection

2.1. High performance liquid chromatography (HPLC)

HPLC is a technique that has been widely used for quantifying ferulic acid from suitable substrates. FAE activity from micro-organisms such as *Aureobasidium pullulans* (*A. pullulans)* NRRL Y-2311-1, *Thermomyces lanuginosus* (*T. lanuginosus*) DSM 5286, an isolate from South Africa, an unidentified *Fusarium* and *Aspergillus* spp. was measured using a method based on the ferulic and coumaric acid liberation [16]. Prior concentration, purification or derivatization was not required in this method. Destarched wheat bran was used as substrate suspended in (3-(*N*-morpholino)) propanesulfonic acid (MOPS) buffer. After the enzyme addition (supernatant obtained from grown micro-organisms) the mixture was incubated, boiling the samples for 3 min before the analysis to stop the reaction. Efficiency (N) of coumaric and ferulic acid separation was 2437, resolution (Rs) and selectivity (α) values were 1.8 and 1.3, respectively. No interference of ferulic and *p*-coumaric acid