

A review on Microbial production of ferulic acid esterase

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Abstract

Ferulic acid esterases (or feruloyl esterases) are a subclass of carboxylic acid esterases that catalyze the hydrolysis of ester bond between polysaccharides and hydroxycinnamic acids (ferulic acid, p-coumaric acid) on the plant cell walls. The release of hydroxycinnamic acids from agroresidues can be achieved by using either of the following methods: chemical release or enzymatic release. The use of enzymatic system in organic synthesis has become a fundamental tool for selective, efficient and economical transformations with the goal of using less aggressive reagents, less organic solvents, lower temperatures and involvement of shorter pathways. Ferulic acid esterases have their potential applications in obtaining ferulic acid from agro-industrial waste materials such as those produced by milling, brewing, and sugar industries. The prospect of broad applications of ferulic acid esterases has fueled much interest in these enzymes, as shown by the increasing number of FAEs discovered in microbial organisms in recent years. This review focuses on the ferulic esterase enzyme isolation and its purification from the culture medium. Extensive search for novel ferulic acid esterase producing microorganisms, by speculating and exploring different environmental niches for their isolation, need an imperative examination. Research is essentially required in order to search for novel ferulic acid esterase producing strain.

Keywords

Ferulic acid esterase, Biotransformation, Screening.

I. INTRODUCTION

The application of FAEs has fuelled much of interest in the enzyme. Microbial enzymes are often more useful than the enzymes released from plant because of the great variety of catalytic activities available, the high yield possible and ease of genetic manipulation. Therefore, it demands for precise, rapid and facile concrete assay method that will benefit in reducing the effort and time required to screen the FAE producers. Many quantitative methods have been developed for screening and detection of FAE activity. These methods include agar plate assay method, spectrophotometric method, capillary zone electrophoresis, gas chromatography and high performance liquid chromatography (Donaghy *et al.* 1998; Donaghy and McKay 1995; Borneman *et al.* 1990; Castanares *et al.* 1992; MacKenzie and Bilous 1988). These all approaches for detection of FAE activity are time consuming, require exorbitant solvents and buffers. Thus, there is a need for a novel detection method which helps to screen out the FAE producers under economic environment within a few hours of culture inoculation.

1. Screening of ferulic acid esterase producing microorganisms

A screening assay method has been developed by Donaghy and McKay (1995) for the detection of phenolic acid esterases using agar medium, but this method experiences some drawbacks like agar medium does not always allow the growth of microorganisms and once the pH sensitive dye is flooded in inoculation plate, it can't be inoculated further. There is another assay protocol which has been developed by Wu *et al.* (2011), based on the pH shift of phenol red. pH shift changed the color from red to yellow because of formation of acid in the reaction, this protocol is not that efficient. Donaghy *et al.* (1998) screened 80 *Bacillus* strains using agar plate supplemented with ethyl ferulate (dissolved in 10% w/v dimethylformamide or DMF) in plate pouring stage as we have used in our study. FAEs were first detected in culture of *Streptomyces olivochromogenes* releasing FA from wheat bran (Mackenzie *et al.* 1987), after which a number of microbes have been isolated and characterized with FAE activity such as *Aspergillus awamori* VTT-D-71025, *Aspergillus nidulans*, *Bacillus subtilis* NCIMB11034, *Clostridium stercoarium* NCIMB 11754 and *Penicillium brasilianum* IBT 20888 (Gong *et al.* 2013; Shin and Chen 2007; Donaghy *et al.* 1998, 2000; Kroon *et al.* 2000) (Table 1).

Table 1 list of reported ferulic acid esterase producing microorganisms (bacteria and fungi).

Microorganisms	FAE	References
<i>Schizophyllum commune</i> ATCC 38548	FAE	Johnson <i>et al.</i> 1989
<i>Fibrobacter succinogenes</i> S851	FAE	McDermid <i>et al.</i> 1990
<i>Aspergillus oryzae</i> VTTD 85248	FAE	Tenkanen <i>et al.</i> 1991
<i>Aspergillus phoenicis</i> IMI 211395	FAE	Smith <i>et al.</i> 1991
<i>Aspergillus terreus</i> VTT-D-82209	FAE	Tenkanen <i>et al.</i> 1991
<i>Neocallimastix</i>	FAE-I, II	Borneman <i>et al.</i> 1992
<i>Panicillium pinophilum</i> CMI 87160ii	FAE	Castanares <i>et al.</i> 1992
<i>Aspergillus niger</i> I-1472	FAE	Faulds <i>et al.</i> 1993
<i>Pseudomonas fluorescens</i>	FAED	Ferreira <i>et al.</i> 1993
<i>Aspergillus niger</i>	FAE-I, II	Kroon <i>et al.</i> 1996
<i>Aspergillus niger</i>	FAE-B	Kroon <i>et al.</i> 1996
<i>Aspergillus tubingensis</i>	FAE-A	de Vries <i>et al.</i> 1997
<i>Penicillium expansum</i>	Possible B	Donaghy <i>et al.</i> 1997
<i>Aspergillus awamori</i> IFO4033	FAE	Koseki <i>et al.</i> 1998
<i>Bacillus substilis</i> NCIMB11034	FAE	Donaghy <i>et al.</i> 1998
<i>Lactobacillus fermentum</i> NCFB1751	FAE	Donaghy <i>et al.</i> 1998
<i>Clostridium stercorarium</i> NCIMB 11754	FAE	Donaghy <i>et al.</i> 2000
<i>Orpinomyces</i> sp PC-2	FAEA	Blum <i>et al.</i> 2000
<i>Penicillium finiculosum</i>	FAEB	Kroon <i>et al.</i> 2000
<i>Penicillium funiculosum</i> IMI-134756	FAE	Kroon <i>et al.</i> 2000
<i>Streptomyces olivochromogenes</i>	FAEA, B, C	Donaghy <i>et al.</i> 2000
<i>Aspergillus pullulans</i> NRRLY 2311-1	FAE	Rumbold <i>et al.</i> 2003
<i>Aureobasidium pullulans</i>	FAE-B	Rumbold <i>et al.</i> 2003
<i>Fusarium proliferatum</i>	FAEB	Rumbold <i>et al.</i> 2003
<i>Neurospora crassa</i>	FAEB, D	Crepin <i>et al.</i> 2003
<i>Streptococcus thermophile</i> ATCC 34628	FAE	Topakas <i>et al.</i> 2003
<i>Streptomyces avermitilis</i> CECT 3339	FAE	Bartolome <i>et al.</i> 2003
<i>Fusarium oxysporum</i>	FoFAE-A, B	Nikos <i>et al.</i> 2004
<i>Fusarium oxysporum</i> F3	FAE	Topakas <i>et al.</i> 2004
<i>Neurospora crassa</i>	FAE-I (FAEB)	Crepin <i>et al.</i> 2004
<i>Sporotrichum thermophile</i>	StFAEA	Topakas <i>et al.</i> 2004
<i>Talaromyces stipitatus</i>	TsFAEA, B	Garcia <i>et al.</i> 2004
<i>Aspergillus awamori</i> VTT-D-71025	FAE	Mathew <i>et al.</i> 2005
<i>Aspergillus flavipes</i>	FAE	Mathew <i>et al.</i> 2005
<i>Aspergillus niger</i>	FAE-A (III)	Faulds <i>et al.</i> 2005
<i>Sporotrichum thermophile</i>	StFAEA, B, C	Vafiadi <i>et al.</i> 2005
<i>Streptomyces avermitilis</i> UAH 30	FAE	Faulds <i>et al.</i> 2005
<i>Aspergillus niger</i> NRRL3	FAE	Shin and Chen 2006
<i>Fusarium proliferatum</i> NRRL 26517	FAE	Shin and Chen 2006
<i>Fusarium verticillioides</i> NRRL 26517 JSC-3	FAE	Shin and Chen 2006
<i>Aspergillus nidulans</i>	FAE-B	Shin and Chen 2007
<i>Streptomyces</i> sp. S10	FAE	Mukherjee <i>et al.</i> 2007
<i>Streptomyces tendae</i>	FAE	Ferreira <i>et al.</i> 2007
<i>Aspergillus niger</i> CFR 1105	FAE	Shyamala <i>et al.</i> 2009
<i>Aspergillus niger</i>	FAE	Ou <i>et al.</i> 2011
<i>Thermobifida</i> sp.	FAE	Huang <i>et al.</i> 2011
<i>Chrysosporium lucknowense</i> C1	FAE	Kuhnel <i>et al.</i> 2012
<i>Aspergillus flavus</i>	FAE	Zhang <i>et al.</i> 2013
<i>Aspergillus usamii</i>	FAE	Gong <i>et al.</i> 2013
<i>Pleurotus eryngii</i>	FAE	Nieter <i>et al.</i> 2014
<i>Mucor hiemalis</i> NCIM 837	FAE	Singh <i>et al.</i> 2015

2. Optimization of process parameters for ferulic acid esterase production

Bioprocess development is an important step for any biotechnological process considering the benefit. It is crucial for large scale production of FAE and it is the primary step towards its commercialization. It may be considered as an important step for cost reduction and increased yield. Industrial scale production of FAE has not been yet successfully achieved due to its low production yield, high recovery and purification cost. Optimization of culture conditions could be one way to increase the yield and development of an economically feasible production process.

Process parameters like pH and temperature have been observed to affect the FAE production. The studies carried on FAE production showed that the optimal pH and temperature for maximum activity ranges from 5.0 to 7.0 and 30 to 60 °C respectively. *Streptomyces olivochromogenes* NRCC 2258 showed 2350 mU/mg FAE activity at 37 °C (Johnson *et al.* 1989) whereas *Sporotrichum thermophile* ATCC 34628 (Topakas *et al.* 2003) showed 156 mU/mg FAE activity at 50 °C. Other microorganisms such as *Schizophyllum Commune* ATCC 38548 (MacKenzie *et al.* 1988), *Aspergillus awamori* IFO4 033 (Koseki *et al.* 1998), *Aspergillus niger* VTTD-77050 (Tenkanen *et al.* 1991), *Bacillus subtilis* ATCC 7661 (Donaghy *et al.* 1998), *Clostridium stercorarium* NCIMB 11754 (Donaghy *et al.* 2000), *Fusarium proliferatum* NRRL 26517 (Shin and Chen 2006) showed their maximum FAE activity at 30 °C.

In addition to the parametric conditions carbon and nitrogen sources are also very essential media components which are required for proper growth and metabolism of the microorganisms, thus, these are required in appropriate amount in media for the enzyme production. Different types of carbon and nitrogen sources have been used by different authors for FAE production (Table 2). *Aspergillus niger* favored FAE production with sugar beet pulp at a concentration of 1.5% (*v/v*) whereas brewer's spent grain is reported as the most suitable natural carbon source for FAE production from *Lactobacillus acidophilus* K1 (2.64 ± 0.06 U) and *Lactobacillus rhamnosus* (Szwajgier *et al.* 2011). Other agroresidues such as rice bran, corn pectin and german wheat bran are also reported for FAE production (Szwajgier *et al.* 2011). Topakas *et al.* (2007) and Wong *et al.* (2013) reported that complex carbon sources viz. wheat bran and sugar beet pulp favors FAE production.

Kumar *et al.* (2011) examined different carbon sources which includes D-glucose, D-fructose, sucrose, lactose, maltose, sorbitol, mannitol, maize starch, and glycerol at 0.5% (*w/v*) concentration in his study, for FAE production from *Aspergillus terreus* GA2 strain and observed potato starch (860 U/gds) as the most effective carbon source for FAE production.

Medium optimization or parametric optimization is conducted either by "one variable at a time" or by using different statistical tools such as response surface methodology (RSM). RSM is superior as it exposes all the possible interaction between variables. The "one-variable-at-a-time" approach is subsidiary for estimating opportune operational intervals for consequential inhibitory/stimulatory variables prior to conducting RSM. Optimization of culture conditions by the one factor at a time is not only time consuming but also very often leads to an incomplete understanding of the system, resulting in confusion and failure of predictive replication. To overcome these inhibitions, RSM is employed more often and this technique explores the interactions between numerous illustrative variables and one or more response variables. RSM was introduced by Box and Wilson in 1951. It is an accumulation of statistical techniques for designing experiments, building blocks, assessing the effects of factors probing for the optimal conditions. It has been prosperously utilized in the optimization of bioprocesses (Cui *et al.* 2009; Sarabia and Ortiz 2009; Shukla *et al.* 2007; Chandrika and Fereidoon 2005; Kristo *et al.* 2003; Wejse *et al.* 2003).

The classical quadratic designs fall into two categories: Box-Behnken designs and Box-Wilson central composite designs. The Box-Behnken design (Box and Behnken 1960) is an independent quadratic design in which it does not contain an embedded factorial or fractional factorial design. In this design the treatment amalgamations are at the midpoints of edges of the process space and at the center. These designs are rotatable and require three levels of each factor. The designs have circumscribed capability for orthogonal blocking compared to the central composite designs. It accommodates as a popular cull to fit a second-order replication surface model with good prognostication variance and has been widely utilized in consummately randomized experiments, split-plot experiments and within the robust parameter design setting (Ferreira *et al.* 2007).

A Box-Wilson Central Composite Design, commonly called a central composite design, contains an imbedded factorial or fractional factorial design with center points that is augmented with a group of 'star points' that sanction estimation of curvature. Statistical optimization of FAE from selected microorganisms has resulted in incremented activity as well as productivity. The mathematical dependences obtained are utilized for presage of the optimum values of the independent variables ascertaining the maximum enzyme activity (Zhang *et al.* 2010; Katapodis *et al.* 2007; Bocchini *et al.* 2002; Techapun *et al.* 2002). The statistical implement has been efficiently utilized for optimization of culture conditions (Chapla *et al.* 2010; Jatinder *et al.* 2005) as well as optimization of media components (Fang *et al.* 2010; Dobrev *et al.* 2006).

Table 2 different studies showing microorganism, carbon sources, culture time and activity obtained.

Microorganism	Carbon source	Culture time	FAE activity	Reference
<i>Aspergillus niger</i> NRCC 401127	De-starched wheat bran	4 d	10,580 mU/mg	Johnson <i>et al.</i> 1989
<i>Streptomyces</i> C 254	De-starched wheat bran	3 d	80 mU/ml	Johnson <i>et al.</i> 1989
<i>Streptomyces olivochromogenes</i> NRCC 2258	De-starched wheat bran	3 d	1200 mU/ml	Johnson <i>et al.</i> 1989
<i>Orpinomyces</i> PC 1	Coastal Bermuda grass + Sisal	5 d	220	Borneman <i>et al.</i> 1990
<i>Piromyces</i> MC 1	Coastal Bermuda grass + Sisal	5 d	560 mU/ml	Borneman <i>et al.</i> 1990
<i>Aspergillus foetidus</i> VTTD 71002	Wheat straw	7 d	12 mU/ml	Tenkanen <i>et al.</i> 1991
<i>Aspergillus niger</i> VTTD 77050	Solka-Floc cellulose	7 d	132 mU/mg	Tenkanen <i>et al.</i> 1991
<i>Aspergillus oryzae</i> VTTD 85248	Wheat bran	7 d	72 mU/mg	Tenkanen <i>et al.</i> 1991
<i>Trichoderma reesei</i> QM 9414	Meadow fescue grass + Glucose	7 d	3000 mU/mg	Smith <i>et al.</i> 1991
<i>Penicillium pinophilum</i> CMI 87160ii	Wheat bran+Oat spelt	12 d	156 mU/ml	Castanares <i>et al.</i> 1992
<i>Aspergillus niger</i> CBS 120.49	Sugar beet pulp	4 d	10 mU/ml	Faulds <i>et al.</i> 1994
<i>Aspergillus awamori</i> IFO 4033	De-starched wheat bran-SFO	3 d	6900 mU/ml	Koseki <i>et al.</i> 1998
<i>Bacillus subtilis</i> ATCC 7661	Methyl ferulate	1 d	1.30 mU/ml	Donaghy <i>et al.</i> 1998
<i>Bacillus subtilis</i> FMCCDL 1	Methyl ferulate	1 d	19.90 mU/ml	Donaghy <i>et al.</i> 1998
<i>Bacillus subtilis</i> NCIMB 3610	Methyl ferulate	1 d	3.40 mU/ml	Donaghy <i>et al.</i> 1998
<i>Clostridium stercorarium</i> NCIMB 11754	De-starched wheat bran	3 d	3.40 mU/ml	Donaghy <i>et al.</i> 2000
<i>Penicillium funiculosum</i> IMI 134756	Sugar beet pulp	6 d	120 mU/mg	Kroon <i>et al.</i> 2000
<i>Aspergillus flavipes</i>	Wheat bran	5 d	33,180 mU/mg	Bartolome <i>et al.</i> 2003
<i>Aspergillus niger</i> 1-1472	Corn bran	5 d	-	Crepin <i>et al.</i> 2003
<i>Aspergillus niger</i> CS 180	Sugar beet pulp	5 d	10.30 mU/mg	Crepin <i>et al.</i> 2003
<i>Aureobasidium pullulans</i> NRRLY 23311-1	Birchwood xylan	2.5 d	347 mU/mg	Rumbold <i>et al.</i> 2003
<i>Fusarium oxysporum</i> F3	De-starched wheat bran	3 d	98 mU/mg	Topakas <i>et al.</i> 2003
<i>Neurospora crassa</i> STA(74 A)	Wheat bran	3 d	9000 mU/ml	Crepin <i>et al.</i> 2003
<i>Sporotrichum thermophile</i> ATCC 34628	Wheat straw	7 d	156 mU/mg	Topakas <i>et al.</i> 2003
<i>Streptomyces avermitilis</i> CECT 3339	De-starched wheat bran	2 d	16.80 mU/mg	Faulds <i>et al.</i> 2003
<i>Talaromyces stipitatus</i> CBS 375.48	Wheat bran	7d	27 mU/mg	Garcia-Conesa <i>et al.</i> 2004
<i>Aspergillus niger</i> NRRL3	Corn bran	5 d	13.90 mU/mg	Shin and Chen 2006
<i>Fusarium proliferatum</i> NRRL 26517	Corn bran	5 d	33.46 mU/mg	Shin and Chen 2006
<i>Penicillium brasilianum</i> IBT 20888	Brewer's spent grain	8 d	1542 mU/ml	Panagiotou <i>et al.</i> 2006
<i>Streptomyces avermitilis</i> UAH 30	De-starched wheat bran	4 d	1.75 mU/ml	Wong <i>et al.</i> 2006

<i>Humicola grisea</i>	Brewers' spent grain	>8 d	0.47 ± 0.01 mU/g	Mandalari <i>et al.</i> 2008
<i>Humicola grisea</i>	Wheat bran	10 d	0.33 ± 0.00 mU/g	Mandalari <i>et al.</i> 2008
<i>Aspergillus niger</i> CFR 1105	Wheat bran	>4 d	32.5 mU/g	Hegde <i>et al.</i> 2009
<i>Bifidobacterium animalis</i> Bi30	Methyl- <i>p</i> -Coumarate	36 h	14.95 mU/g	Szwajgier <i>et al.</i> 2010
<i>Bifidobacterium longum</i> KN29	Methyl- <i>p</i> -Coumarate	36 h	13.53 mU/g	Szwajgier <i>et al.</i> 2010
<i>Aspergillus niger</i> ATCC 16404	Wheat bran	72 h	3.8 ± 0.12 mU/g	Ou <i>et al.</i> 2011
<i>Aspergillus terreus</i> GA2	Potato starch	7 d	860 U/gds	Kumar <i>et al.</i> 2011
<i>Lactobacillus acidophilus</i> K1	Methyl Ferulate	60 h	23.32 ± 0.05 mU/g	Szwajgier <i>et al.</i> 2011
<i>Lactobacillus rhamnosus</i> OXY	Methyl- <i>p</i> -Coumarate	36 h	3.64 ± 0.84 mU/g	Szwajgier <i>et al.</i> 2011

3. Ferulic acid esterase purification

The various purification procedures have been employed for obtaining pure FAE enzyme from the crude extract obtained from different bacteria, fungi and plant sources. PeFAE from *Pleurotus eryngine* has been purified using cation exchange hydrophobic interaction (HIC) and finally size exclusion chromatography (SEC) (Nieter *et al.* 2014). Kuhnel *et al.* (Kuhnel *et al.* 2012) purified an extracellular enzyme FeaA2 from culture filtrate produced from *Chrysosporium lucknowense* C1 and FAEB2 by hydrophobic interaction chromatography followed by anion exchange chromatography using an AKTA explorer 100 preparative system (GE healthcare) (Table 3).

Ammonium sulphate precipitation (ASP) method is the most common type of protein precipitation techniques. Different types of salts such as sodium sulphate and ammonium sulphate are widely used to precipitate out proteins. Among which, ammonium sulphate is the most widely used salt for the precipitation of proteins as it is inexpensive, highly soluble and easily available in highest purity level and moreover in most of the cases it does not denature proteins. Ammonium sulphate can be used for precipitation of protein to approx. 90% saturation or for differential precipitation level of proteins using different saturation of salts. Various reports are available where FAE has been purified using ammonium sulphate precipitation method followed by chromatographic methods like AnFAE and AaFAE (Hegde and Muralikrishna 2009 and Koseki *et al.* 1997).

All chromatographic techniques depend on differences in the distribution of the sundry compounds in the applied mixture between the mobile phase and the stationary phase. Ion exchange chromatography is the most popular method for the purification of proteins and other charged biomolecules based on the reversible interaction between the molecule and oppositely charged chromatography medium. This chromatography helps to speed biomolecules with even a small difference in their surface charge by choosing the optimal buffer pH and ion exchange. DEAE- anion exchange resins have been used for the purification of most of the proteins (Wu *et al.* 2011).

Thin layer chromatography is simple, rapid, and inexpensive method for the separation, tentative identification, and visual semi-quantification of a wide variety of substances. Gel filtration chromatography is a separation technique used to separate proteins on the basis of shape and their size, additionally applicable for the separation of molecular weight of proteins. The main aim of the gel filtration chromatography is fractionation of proteins. Smaller molecules incline to spend more time in the matrix, whereas the higher molecular weight molecule elute from the column before the minute molecules. Matrixes used to purify esterases from the microbes are sepharose, sephacryl, and sephadex etc. FAE was purified from *Lactobacillus acidophilus* by gel filtration chromatography utilizing sephacryl S-300 (Szwajgier *et al.* 2010)

Table.3 comparison of consecutive steps of ferulic acid esterase purification from Individual microorganisms

Microorganisms	Purification steps	FAE activity (U/mg protein)	Yield (%)	Purification (fold)	References
<i>Aspergillus awamori</i> IFO4033	Culture supernatant	0.6	100	1.0	Koseki <i>et al.</i> 1997
	Ammonium sulphate precipitation fractions (40 ± 80%)	1.1	74.5	1.8	
	DEAE-5PW	2.6	49.0	4.3	
	G-2000SW	4.3	38.7	7.2	
	Phenyl-5PW	13.2	20.3	22.0	

<i>Fusarium oxysporum</i>	Culture filtrate	1.7	100.0	1.0	Topakas <i>et al.</i> 2003
	SP-Sepharose	2.3	80.0	1.4	
	1st t-butyl-HIC	4.3	46.0	2.5	
	2nd t-butyl-HIC	5.4	23.0	3.2	
	Sephacryl S-200	22.5	21.0	13.2	
<i>Aspergillus niger</i> CFR1105	Crude	3.23	100	1.0	Hegde and Muralikrishna 2009
	Ammonium sulphate precipitation fraction (30-60%)	11.9	66.7	3.7	
	DEAE cellulose, FAE-1 and FAE-2 respectively	21.5, 30.1	10.0, 8.4	7.6, 9.3	
	Sephacryl 100-HR, FAE-1 and FAE-2 respectively	35.9, 80.0	4.3, 2.5	11.11, 24.8	
<i>Lactobacillus acidophilus</i> K1	Crude enzyme solution	1.58	100.00	1.0	Szwajgier <i>et al.</i> 2010
	Ultrafiltration	1.6	5.82	1.01	
	Dialysis	7.81	12.62	4.94	
	Vacuum concentration	13.2	11.39	8.35	
	Ion exchange chromatography	17.62	5.45	11.15	
	Size exclusion chromatography, Sephacryl S-300	48.97	2.25	30.99	

4. Purity criteria of Ferulic acid esterase

Different methods are in use to assure the homogeneity of enzyme which includes Polyacrylamide gel electrophoresis (PAGE), which separates molecules according to their size and charge. During electrophoresis there is an interaction of samples, gel matrix buffers, and electric current resulting in separate bands of individual molecules. Empirically the pore size providing optimum resolution for proteins is that which results in the relative mobility (Rf) value between 0.55-0.6. Rf values for categorical proteins is calculated as follows:

$$R_f = \frac{\text{Distance migrated by protein of interest}}{\text{Distance migrated by ion front}}$$

Almost all analytical electrophoresis of proteins are carried out with SDS PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis). SDS PAGE was carried out under conditions that ascertain dissociation of proteins into their individual polypeptide subunits and that minimize aggregation. The Laemmli buffer system is a discontinuous buffer system that incorporates SDS, a vigorously anionic detergent in buffer (Laemmli 1970). The proteins are separated according to their molecular weight, making this system astronomically subsidiary for calculating molecular weight and to assure purity.

5. Physiochemical properties of ferulic acid esterases

FAEs have been purified and characterized from different microorganisms named *Penicillium brevicompactum*, *Talaromyces stipitatus* CBS 375.48, *Lactobacillus acidophilus* IFO13951, *Chrysosporium lucknowense* C1 and *Pleurotus eryngii* (Nieter *et al.* 2014; Kuhnel *et al.* 2012; Garcia *et al.* 2004; Donaghy *et al.* 1995). FAE activity was also observed in various *Aspergillus* species such as *Aspergillus niger*, *Aspergillus awamori*, *Aspergillus flavipes* and *Aspergillus nidulans* (Debeire *et al.* 2012; Shin and Chen 2007; Koseki *et al.* 2005; Mathew *et al.* 2005; Faulds *et al.* 1995). Purified FAEs show significant variation in physical and chemical characteristics such as isoelectric point, molecular weight and optimal hydrolytic reaction conditions. The molecular weight of the purified FAE ranges from the 11-210 kDa. FAEA1 and FAEB2 from the *Chrysosporium lucknowense* C1 have been reported with molecular weight (determined by SDS-PAGE) of 29, 36 kDa and isoelectric point 5.5, 5.2, respectively (Kuhnel *et al.* 2012). However the FAEA2 from the same organism was reported to have a molecular weight of 33 kDa and PI of 6.0A. FAE from *Aspergillus niger* CS180 is a heavily glycosylated dimer (Kroon *et al.* 1996). FAE from *Aureobasidium pullulans* was found with 48% glycosylation (Rumbold *et al.* 2003). A glycosylated FAE (TsFAEC) has been isolated from the fungus *Talaromyces stipitatus* (Garcia-Conesa *et al.* 2004) (Table 4).

Microbial FAE has a broad range of temperature and pH dependence. They exhibit optimal activities between temperature 30 to 65 °C and pH 4.0 to 8.0. FAEA1 and FAEB2 from *Chrysosporium lucknowense* C1 have been reported to be highly active in the range of 45 to 60 °C and pH 5.0 to 7.0 respectively, whereas FAEA2 was less stable than FAEA1 and FAEB2, showing the optimum temperature in the range of 35 to 40 °C Kuhnel *et al.* 2012. FAE from *Pleurotus eryngii* showed an optimum pH of 5.0 and showed more than 50% residual activity over a broad pH range from 3.5 to 7.0 and optimum temperature for maximum enzyme activity was 50 °C. The PeFAEA showed good activity at a temperature ranging from 40-50 °C (Table 5).

Table 4 molecular weight, optimum ph and temperature for ferulic acid esterase released from individual microorganisms

Microorganisms	Enzyme	Enzyme type	Molecular weight (kDa)	pH optimum	Thermo - stability	Reference
<i>Aspergillus oryzae</i>	FAE		30	4.5-6.0	45	Tenkanen <i>et al.</i> 1991
<i>Neocallimastix</i>	FAE		69	5.5-6.8		Borneman <i>et al.</i> 1992
<i>Penicillium pinophilum</i>	FAE		57	6	55	Castanares <i>et al.</i> 1992
<i>Aspergillus niger</i>	FAE	B	29			Faulds and Williamson 1993
<i>Aspergillus niger</i>	FAE	A	36	5	60	de Vries <i>et al.</i> 1997
<i>Aspergillus tubigenensis</i>	FAE	A	36	5	60	de Vries <i>et al.</i> 1997
<i>Penicillium expansum</i>	FAE		65	5.6	37	Donaghy <i>et al.</i> 1997
<i>Aspergillus awamori</i>	AwFAE	A	37	7	45	Koseki <i>et al.</i> 1998
<i>Aspergillus nidulans</i>	AnFAE	B	56	7	45	Donaghy <i>et al.</i> 1998
<i>Aureobasidium pullulans</i>	FAE	B	210	6.7	60	Donaghy <i>et al.</i> 1998
<i>Clostridium stercorarium</i>	FAE	C, D	33	8	65	Donaghy <i>et al.</i> 2000
<i>Penicillium funiculosum</i>	FAE	B	53			Kroon <i>et al.</i> 2000
<i>Streptomyces olivochromogenes</i>	FAE		29	5.5	30	Donaghy <i>et al.</i> 2000
<i>Talaromyces stipitatus</i>	TsFAE	A, B, C	35, 35, 65	6.0-7.0	60	Malherbe <i>et al.</i> 2002
<i>Aspergillus sp.</i>	FAE		42	4	50	Record <i>et al.</i> 2003
<i>Fusarium proliferatum</i>	FAE	B	31	6.5-7.5	56	Rumbold <i>et al.</i> 2003
<i>Neurospora crassa</i>	FAE	B, D	35	6	55	Crepin <i>et al.</i> 2003
<i>Talaromyces stipitatus</i>	TsFAE	C	66	6.0-7.0	60	Crepin <i>et al.</i> 2003b
<i>Fusarium oxysporum</i>	FoFAE	A, B	27, 31	7	45-55	Nikos <i>et al.</i> 2004;
<i>Neurospora crassa</i>	FAE	D	32			Crepin <i>et al.</i> 2004a
<i>Sporotrichum thermophile</i>	StFAE	C	23	6	55	Topakas <i>et al.</i> 2004
<i>Talaromyces stipitatus</i>	TsFAE	A	35			Garcia-conesa <i>et al.</i> 2004
<i>Talaromyces stipitatus</i>	TsFAE	B	35			Garcia-conesa <i>et al.</i> 2004
<i>Sporotrichum thermophile</i>	StFAE	A, B, C	33, 23	6	55	Vafiadi <i>et al.</i> 2005
<i>Aspergillus nidulans</i>	FAE	B	130	7	45	Shin and Chen 2007
<i>Aspergillus niger</i>	FAE	A, B	36	5	55	Zheng <i>et al.</i> 2007;
<i>Aspergillus oryzae</i>	AoFAE	B	61	6	55	Koseki <i>et al.</i> 2009
<i>Aspergillus oryzae</i>	AoFAE	C	75	6	60	Koseki <i>et al.</i> 2009

TABLE 5 OPTIMUM PH AND TEMPERATURE FOR FERULIC ACID ESTERASE ACTIVITY

Microorganism	Optimal pH	Optimal Temperature (°C)	Reference
<i>Aspergillus oryzae</i>	4.5–6.0	Upto 45	Tenkanen <i>et al.</i> 1991
<i>Aspergillus niger</i> CBS 120.49	5.0	55–60	Faulds and Williamson 1994
<i>Streptomyces avermitilis</i> CECT 3339	6.0	50	Garcia <i>et al.</i> 1998
<i>Fusarium oxysporum</i>	7	45	Topakas <i>et al.</i> 2004
<i>Bifidobacterium animalis</i> Bi30	6.3	37	Szwajgier <i>et al.</i> 2010
<i>Bifidobacterium catenulatum</i> KD 14	6.3	37	Szwajgier <i>et al.</i> 2010
<i>Aspergillus niger</i> (ATCC 16404)	-	36	Ou <i>et al.</i> 2011
<i>Lactobacillus acidophilus</i> K ₁	6.3	37	Szwajgier <i>et al.</i> 2011
<i>Lactobacillus rhamnosus</i> E/N	6.5	37	Szwajgier <i>et al.</i> 2011
<i>Lactobacillus rhamnosus oxy</i>	6.3	37	Szwajgier <i>et al.</i> 2011
<i>Aspergillus flavus</i> CBE 332.1	5.5	58	Zhang <i>et al.</i> 2013
<i>Pleurotus eryngii</i>	5.0	50	Nieter <i>et al.</i> 2014
<i>Mucor hiemalis</i> NCIM 837	5.0	30	Singh <i>et al.</i> 2015

CONCLUSION

Microbial production of ferulic acid esterase as biocatalyst for release of ferulic acid from agroresidues, suggest that these microorganisms may be useful in ferulic acid esterase production on a large scale. FAEs are highly applicable in pharmaceutical, food and cosmetic industries. Furthermore, the purified enzyme may contribute significantly to the production of ferulic acid, which is applied in production of vanillin; the most flavoring agent. Recently, due to advances in biology and computational approaches, the information can be used to identify and characterize the gene, which are involved in the release of ferulic acid esterase. This will offer new opportunities on a metabolic level to develop more efficient industrial process for production of ferulic acid esterase.

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AUTHOR CONTRIBUTION

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Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors

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