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Standard Review

The large-scale use of feruloyl esterases in industry

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Presently industrial enzyme companies sell enzymes for a wide variety of applications. The estimated value of world enzyme market is presently about US $ 4.3 billion and it has been forecasted to grow to almost US $ 5.1 billion by 2009. Detergents (37%), textiles (12%), starch (11%), baking (8%) and animal feed (6%) are the main industries; totally these industries use about 74% of industrially produced enzymes. Enzymes are also indirectly used in biocatalytic processes involving living or dead and permeabilized microorganisms. This review concentrates on the use of isolated feruloyl esterases enzyme preparations in large scale and speciality applications and chemical manufacturing.

Key words: Feruloyl esterases, industry, biotechnology.

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INTRODUCTION

There is an increased interest in feruloyl esterases (FAEs) production which has considerable roles in biotechnological processes for various industrial and medicinal applications. As reported by us in 2007 (Fazary et al., 2007, 2008), the quantum of research carried out in FAEs has incre-ased dramatically since 1990. For example, during 1990 – 2000 and 2001–mid 2008, the average number of re-fereed papers involving FAEs as a major component was 24 and 60, respectively. It is interesting to note that the dramatic increase in publications concerning to FAEs between 2001 (four papers) and mid 2008 (20 papers) coincides with the most recent discoveries on isolation, purification, and characterization of FAEs including fungal and bacterial FAEs (Figure 1).

Results of search for feruloyl esterases in US Patent Collection data base, US Patent applications, European Parliament documents (EP) and in World Intellectual Property Organization-Patent Cooperation Treaty (WIPO-PCT) reveal 29, 50, 11, and 75 patents, respectively (Figure 2) (Table 1).

There was also remarkable increase in the number of publications concerned with ferulic acid (the related compound to FAEs). The average number of documents was 242 paper for the period (1990 – 2000), while for the period 2001–2008, the average number was 575 paper

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Table 1. Summary of Patents on feruloyl esterases

No.	Patent No.	Patent Title
1	US20080145903	Process of Producing Fermentation Products
2	US20080138872	Processes for Producing Fermentation Products
3	US20080138862	Enzymatic Hydrolysis of Biomasses Having a High Dry Matter (Dm) Content
4	US20080138856	Enzymatic Enantioselective Ester or Amide Hydrolysis or Synthesis
5	WO/2008/064025A2	Cloning and sequencing of the ferulate esterases gene from lactobacillus buchneriln4017
6	WO/2008/061982A1	Novel Method to Reduce Compounds Involved in Maillard Reactions in Thermally Processed Plant-Based Food Products
7	US20080118491	Xylanase gene sequences from the genomic DNA of unpurified rumen microorganisms
8	7374925	*Penicillium funiculosum* mutant strain
9	US20080115241	Cloning and Sequencing of the Ferulate Esterase Gene from Lactobacillus Buchneri LN4017
10	7371423	Method for preparing flour doughs and products made from such doughs using lipase
11	US20080096253	Methods of making nanotechnological and macromolecular biomimetic structures
12	WO/2008/039353A2	Novel pectate lyase and method of. use for bio-scouring
13	US20080076821	Intranasally administering curcumin prodrugs to the brain to treat alzheimer's disease
14	WO/2008/028300A1	Oral polymeric membrane feruloyl esterase producing bacteria formulation
15	US20080050321	Formulations with Feruloyl Glycerides and Methods of Preparation
16	US20080026101	Food products
17	US20080025921	Conductance of Improperly Folded Proteins Through the Secretory Pathway And Related Methods For Treating Disease
18	WO/2008/008070A2	Construction of highly efficient cellulase compositions for enzymatic hydrolysis of cellulose
19	WO/2008/003090A2	Formulations with Feruloyl Glycerides and Methods of Preparation

Table 1 Continued

21	US20070254336	Transcription Factors
22	WO/2007/115723A2	Fusion fungal proteins between plant-wall degrading enzymes and a swollenin and their uses
23	US20070238155	Construction of highly efficient cellulase compositions for enzymatic hydrolysis of cellulose
24	US20070232495	Compositions and methods to add value to plant products, increasing the commercial quality, resistance to external factors and polyphenol content thereof
25	US20070224668	Process for producing 4-vinylguaiacol by biodecaroxylation of ferulic acid
26	US20070202566	Hydrolases, Nucleic Acids Encoding Them And Methods For Making And Using Them
27	WO/2007/092314A2	Esterases and Related Nucleic acids and methods
28	US20070184175	Continuous production of cereal flour and whole-cereal flour for grain-based foods, using a low-moisture precooking
29	US20070179076	Detergent composition
30	WO/2007/076436A2	Continuous production of cereal flour and whole-cereal flour for grain-based foods, using a low-moisture precooking
31	US20070154591	Chewing gum comprising biodegradable polymers and having accelerated degradability
32	EP1309674B1	Stereo-selective esterase from Aspergillus oryzae
33	7226770	Lipolytic enzyme variant
34	WO/2007/055735A2	Hydrolyses, nucleic acids encoding them and methods for making and using them
35	US20070077636	Methods of making compositions comprising a uv-absorbing chromophore
36	US20070072185	Carbohydrate-binding modules of a new family
37	WO/2007/019949A1	Fusion proteins between plant cell-wall degrading enzymes, and their uses
38	US20070037259	Integration of alternative feedstreams for biomass treatment and utilization
39	EP1752533A1	Fusion proteins between plant cell-wall degrading enzymes, and their uses
40	US20070031953	Treatment of biomass to obtain ethanol
41	US20070031919	Treatment of biomass to obtain a target chemical
42	US20070031918	Treatment of biomass to obtain

Table 1 Continued

		fermentable sugars
43	US20070029252	System and process for biomass treatment
44	7172997	Lipolytic enzyme variant
45	WO/2006/136161A2	Amylases for pharmaceutical use
46	WO/2006/136160A2	Proteases for pharmaceutical use
47	WO/2006/136159A2	Lipases for pharmaceutical use
48	EP1726213A1	Soluble coffee product
49	7132589	Manipulation of the phenolic acid content and digestibility of plant cell walls by targeted expression of genes encoding cell wall degrading enzymes
50	US20060229223	Lipolytic enzyme variant
51	7091023	Stereoselective esterase from Aspergillus oryzae
52	US20060035800	Detergent composition
53	WO/2006/003009A2	NEW ESTERASES FROM RUMEN
54	US20060005270	Manipulation of the phenolic acid content and digestibility of plant cell walls by targeted expression of genes encoding cell wall degrading enzymes
55	WO/2005/115445A1	ENZYMES FOR PHARMACEUTICAL USE
56	US20050227344	Mixture obtained from penicillium funiculosu
57	US20050181446	Protein variants having modified immunogenicity
58	EP1555322A1	Lipolytic enzyme variant
59	US20050118665	Methods for conducting assays for enzyme activity on protein microarrays
60	WO/2005/042735A1	A carbohydrate-binding module of a new family
61	WO/2005/040107A2	Methods for Making Simvastatin and Intermediate
62	US20040266883	Conductance of improperly folded proteins through the secretory pathway and related methods for treating disease
63	6828136	Esterase enzymes, DNA encoding esterase enzymes and vectors and host cells incorporating same
64	US20040231060	Methods to enhance the activity of lignocellulose-degrading enzymes
65	WO/2004/081185A2	Methods to enhance the activity of lignocellulose-degrading enzymes
67	US20040152180	Lipolytic enzyme variant
68	WO/2004/053039A2	Detergent composition comprisingEndoglucanase
69	6750051	Compositions and methods for enhancing fiber digestion
70	EP0904396B1	Production of vanillin

Table 1 Continued

71	WO/2004/009804A2	Feruloyl esterases and uses thereof
72	US20040005674	Methods for enzymatic hydrolysis of lignocellulose
73	US20040002136	Transformation system in the field of filamentous fungal hosts
74	US20030236300	Conductance of improperly folded proteins through the secretory pathway and related methods for treating disease
75	6664088	Production of vanillin
76	WO/2003/093420A2	Methods enzymatic hydrolysis of lignocellulose
77	US20030199048	Stereoselective estarase from aspergillus oryzae
78	US20030175384	Method for the extraction of aleurone from bran
79	US20030167511	Production of p-hydroxybenzoic acid
80	US20030149113	Conductance of improperly folded proteins through the secretory pathway and related methods for treating disease
81	6602700	Phenolic acid esterases, coding sequences and methods
82	US20030144165	Lipolytic enzyme variant
83	6589760	Methods of separating a corn fiber lipid fraction from corn fiber
84	6586212	Corn fiber for the production of advanced chemicals and materials: derivatizable cellulose and cellulose derivatives made therefrom
85	WO/2003/049717A2	Conductance of improperly folded proteins through the secretory pathway and related methods for treating disease
86	US20030108642	Penicillium funiculosum strain useful for the production of enzymes
87	WO/2003/046163A2	Multifunctional Caffeic Acid O-methyltransferase
88	6573086	Transformation system in the field of filamentous fungal hosts
89	WO/2003/043411A2	Manipulation of the phenolic acid content and digestibility of plant cell walls
90	6534286	Protein production in Aureobasidium pullulans
91	6534101	Enzymes mixture obtained from Penicillium funiculosum
92	US20030035822	Compositions and methods for enhancing fiber digestion
93	US20030032161	Esterase enzymes, DNA encoding esterase enzymes and vectors and host cells incorporating same

Table 1 Continued

94	US20030024009	Manipulation of the phenolic acid content and digestibility of plant cell walls by targeted expression of genes encoding cell wall degrading enzymes
95	WO/2003/000898A1	Plant genes involved in defence against pathogens
96	WO/2002/068666A1	Manipulation of the phenolic acid content and digestibility of plant cell walls by targeted expression of genes encoding cell wall degrading enzymes
97	WO/2002/055679A2	thermostable lipolytic enzyme variant
98	6388069	Corn fiber for the production of advanced chemicals and materials:arabinoxylan and arabinoxylan derivatives made therefrom
99	6368833	Esterases, DNA encoding therefor and vectors and host incorporating same
100	6365390	Phenolic acid esterases, coding sequences and methods
101	6352845	Corn fiber for the production of advanced chemicals and materials: separation of monosaccharides and methods thereof
102	WO/2002/012472A1	Stereoselective esterase from Aspergillus oryzae
103	WO/2002/002825A1	Method for the production of proteins and polypeptides using fungal cells, in particular Aspergillus vadensis.
104	WO/2001/096578A2	Protein production in Aureobasidium pullulans
105	6323011	Production of vanillin
106	WO/2001/083770A2	Lipolytic enzyme variant
107	WO/2001/083559A2	Production and Use of Protein Variants having modified immunogenicity
108	US20010014467	Production of vanillin
109	WO/2001/042433A2	Talaromyces emersonii Xylanses
110	6245363	Methods of treating plant materials with hydrolytic enzymes isolated from humicola species
111	6232101	Oxidase-promoted gelling of phenolic polymers
112	6137032	Xylanase obtained from an anaerobic fungus
113	WO/2000/020555A2	Transformation system in the field of filamentous fungal hosts
114	WO/2000/017368A1	Orange fruit pectinacetylesterase
115	WO/2000/014243A1	Phenolic acid esterases, coding sequences and methods
116	EP0976838A1	Enzymes mixture

Table 1 Continued

117	WO/2000/004053A1	Purification of Hemicelluloses materials
118	WO/1999/057325A2	Enzyme Miurext
119	5948667	Xylanase obtained from an anaerobic fungus
121	WO/1999/011672A1	Fractionation of Hemicelluloses materials
122	5869720	Transgenic cotton plants producing heterologous peroxidase
123	5824533	Orpinomyces xylanase proteins and coding sequences
124	WO/1998/014594A2	Esterases, DNA encoding therefor and vectors and host incorporating same
125	WO/1997/035999A2	Production of vanillin
126	5608148	Transgenic cotton plants producing heterologous peroxidase
127	5591619	Aureobasidium pullulans xylanase, gene and signal sequence
128	WO/1996/040182A1	Identification of a potent antioxidant from Aloe barbadensis
129	WO/1996/036701A1	Orpinomyces xylanase proteins and coding sequences
130	WO/1996/003440A1	Oxidize-Promoted Gelling of Phenolic Polymers
131	WO/2004/009804A3	Feruloyl Esterases and uses thereof

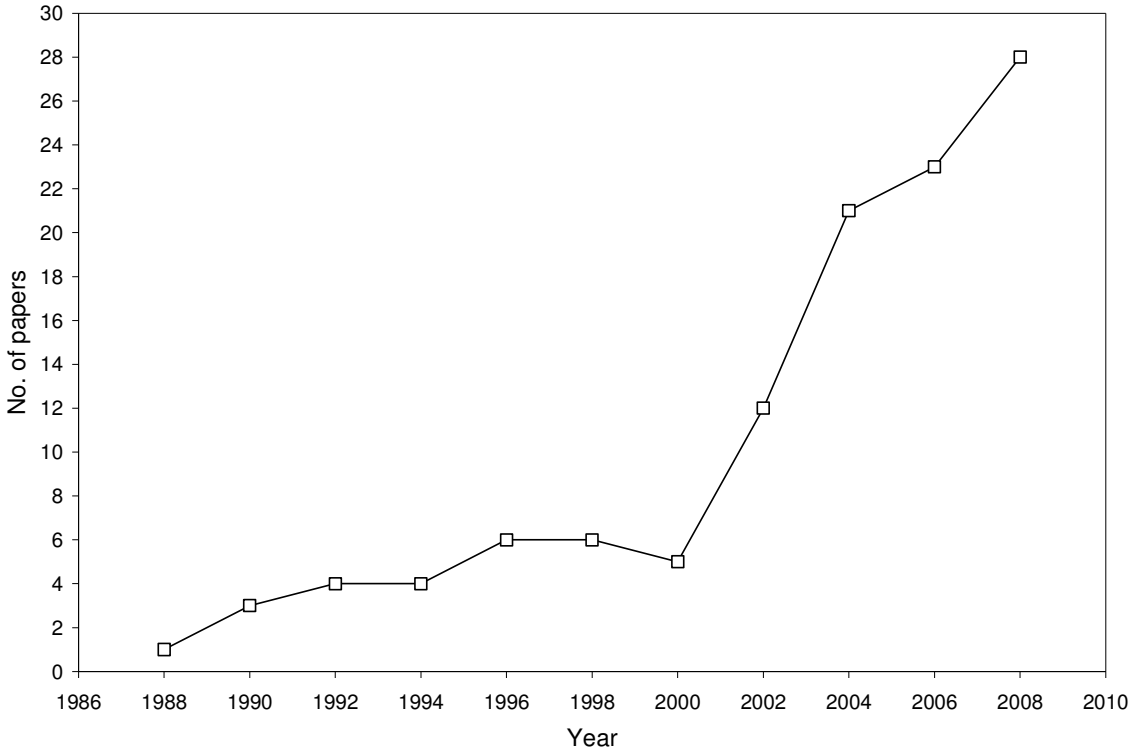


Figure 1. Annual publications on feruloyl esterases

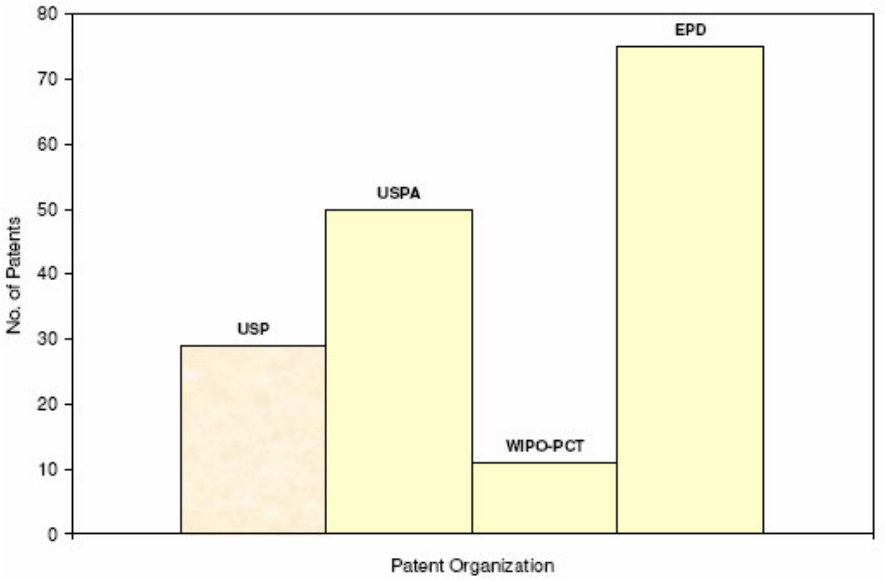


Figure 2. Patents recorded for feruloyl esterases. USP; United States Patent collection data base. USPA; United States Patent Applications data base. EPD; European Parliament documents. WIPO-PCT; World Intellectual Property Organization-Patent Cooperation Treaty.

using the same search engine (Elsevier SDOS) (Figure 3).

In this review, we will try to report concise and complete highlights of the large-scale usage of such kind of enzyme (feruloyl esterases) in both academic research and industry.

WHAT IS FERULOYL ESTERASES?

Recently, it was reported that ferulic acid had been shown to link hemicellulose and lignin. Cross-linking of ferulic acids within cell wall components influences wall properties such as extensibility, plasticity, and digestibility, and limits the access of polysaccharidaes to their substrates (Borneman et al., 1990).

The complete degradation of plant cell wall polymers requires an array of enzymes with different activities. The hydrolysis of hydroxycinnamate esters in plant cell walls is catalyzed by cinnamoyl esterases (CEs; such as cinnamoyl ester hydrolases, feruloyl/p-coumaroyl esterases, and ferulic/p-coumaric acid esterases). CEs are a subclass of the carboxylesterases (EC 3.1.1.1) and characterized by a relatively high activity on various hydroxycinnamate esters (Fazary et al., 2007).

Two enzymes, feruloyl and cinnamoyl esterases (FAE-B), purified from *Aspergillus niger* strains (Fazary et al., 2008), have different physicochemical characteristics and catalytic properties against cinnamoyl model substrates, such as methyl derivatives of hydroxycinnamic esters and soluble feruloylated oligosaccharides derived from plant cell wall (Ralet et al., 1994). These enzymes are exocellular and the corresponding expression is inducible. A new strain of *A. niger* I-1472 was shown to produce numerous polysac-charide-degrading enzymes as well as esterases that released ferulic acid from natural feruloylated oligosac-charides, when grown on sugar beet pulp and maize bran (Hutnan et al., 2000).

FAEs (EC 3.1.1.73), including cinnamoyl esterases and cinnamic acid hydrolases, are a subclass of the carboxylic acid esterases (EC 3.1.1) that play a key physiological role in the degradation of the intricate structure of plant cell wall by hydrolyzing ferulate ester groups involved in the cross-linking between hemicelluloses and between hemicellulose and lignin (Lee et al., 2005). The use of multiple alignments of sequences or domains that show FAE activity, as well as related sequences, helped to construct a neighborhood-joining phylogenic tree (Table 2). The outcome of this genetic comparison supported substrate specificity data and allowed FAEs to be sub-classified into four types, A, B, C, and D (Crepin, et al. 2003a, b, c, 2004; Donaghy et al., 1998, 1995; Faulds et al., 2002, 1997, 1993), based on their substrate specificities towards synthetic methyl esters of hydroxycinnamic acids (ferulic acid, diferulic acids, p-coumaric acid, sinapinic acid, and caffeic acid) for substitutions on the phenolic ring, and on their amino acid sequence identity (protein sequence), indicating an evolutionary relationship among FAEs, acetyl xylan esterases, and certain lipases.

By looking in the Comprehensive Enzyme Information System (http://www.brenda-enzymes.info/), we found that there are only 22 protein database entries in that type of enzyme. Considering the remarkable impact of FAEs in

Table 2. Gene Product Associations to feruloyl esterase activity

Symbol, Full Name	Primary Peptide Sequence	Species	GO Evidence*	Database	Reference
FAEA_ASPAW faeA: Feruloyl esterase A precursor	MKQFSAKHALAVVVTAGHALAASTQGISEDLYTRLVEMATISQAAYADLCNIPSTIIKGEKIYNSQTDINGWILRDDSSKEIITVFRGTGGDTNLQLDTNYTLTPFDTLPQCNGCEVHGGYYIGWVSVQDQVESLVKQQVSQYPDYALTVTGHSLGASLAALTAAQLSATYDNIRLYTFGEPRSGNQAFASYMNDAFQASSPDTTQYFRVTHANDGIPNLPPVEQGYAHGGVEYWSVDPYSAQNTFVCTGDEVQCCEAQGGQGVNNAHTTYFGMTSGACTW	*Aspergillus awamori*	ISS	UniProtKB/Swiss-Prot Q9P979	de Vries st et al., 1997
FAEA_ASPNG faeA: Feruloyl esterase A precursor	MKQFSAKYALILLATAGQALAASTQGISEDLYNRLVEMATISQAAYADLCNIPSTIIKGEKIYNAQTDINGWILRDDTSKEIITVFRGTGSDTNLQLDTNYTLTPFDTLPQCNDCEVHGGYYIGWISVQDQVESLVKQQASQYPDYALTVTGHSLGASMAALTAAQLSATYDNVRLYTFGEPRSGNQAFASYMNDAFQVSSPETTQYFRVTHSNDGIPNLPPADEGYAHGGVEYWSVDPYSAQNTFVCTGDEVQCCEAQGGQGVNDAHTTYFGMTSGACTW	*Aspergillus niger*	IDA	UniProtKB/Swiss-Prot O42807	de Vries st et al., 1997
FAEA_ASPTU faeA: Feruloyl esterase A precursor	MKQFSAKYAIAVVVTAGHALAASTQGISEDLYSRLVEMATISQAAYADLCNIPSTIIKGEKIYNSQTDINGWILRDDSSKEIITVFRGTGSDTNLQLDTNYTLTPFDTLPQCNSCEVHGGYYIGWISVQDQVESLVQQQVSQFPDYALTVTGHSLGASLAALTAAQLSATYDNIRLYTFGEPRSNQAFASYMNDAFQASSPDTTQYFRVTHANDGIPNLPPADEGYAHGVVEYWSVDPYSAQNTFVCTGDEVQCCEAQGGQGVNNAHTTYFGMTSGHCTW	*Aspergillus tubingensis*	ISS	UniProtKB/Swiss-Prot O42815	de Vries st et al., 1997
FAEA_ASPNG faeB: Feruloyl esterase B precursor	MKVASLLSLALPGAALAATDPFQSRCNEFQNKIDIANVTVRSVAYVAAGQNISQAEVASVCKASVQASVDLCRVTMNISTSDRSHLWAEAWLPRNYTGRFVSTGNGGLAGCVQETDLNFAANFGFATVGTNGGHDGDTAKYFLNNSEVLADFAYRSVHEGTVVGKQLTQLFYDEGYNYSYYLGCSTGGRQGYQQVQRFPDDYDGVIAGSAAMNFINLISWGAFLWKATGLADDPDFISANLWSVIHQEIVRQCDLVDGALDGIIEDPDFCAPVIERLICDGTTNGTSCITGAQAAKVNRALSDFYGPDGTVYYPRLNYGGEADSASLYFTGSMYSRTEEWYKYVVYNDTNWNSSQWTLESAKLALEQNPFNIQAFDPNITAFRDRGGKLLSYHGTQDPIISSTDSKLYYRRVANALNAAPSELDEFYRFFQISGMGHCGDGTGASYIGQGYGTYTSKAPQVNLLRTMVDWVENGKAPEYMPGNKLNANGSIEYMRKHCRYPKHNIHTGPGNYTDPNSWTCV	*Aspergillus niger*	IDA	UniProtKB/Swiss-Prot Q8WZI8	de Vries st et al., 2002

various industrial and medical applications, it is anticipated that these crystal structural studies (22 protein database) will provide an initial framework for the rational design of novel enzymes with improved biotechnological potential (Table 3) (Faulds et al., 1997).

Major extent of work on the microbial production of

FAEB_NEUCR fae-1, B22K18.040, NCU09491: Feruloyl esterase B precursor	MLPRTLLGLALTAATGLCASLQQVTNWGSN PTNIRMYTYVPDKLATKPAIIVALHGCGGT APSWYSGTRLPSYADQYGFILIYPGTPNMSN CWGVNDPASLTHGAGGDSLGIVAMVNYTI AKYNADASRVYVMGTSSGGMMTNVMAATY PEVFEAGAAYSGVAHACFAGAASATPFSPN Q TCARGLQHTPEEWGNFVRNSYPGYTGRRP RMQIYHGLADNLVYPRCAMEALKQWSNVLG V EFSRNVSGVPSQAYTQIVYGDGSKLVGYMG AGVGHVAPTNEQVMLKFFGLIN	*Neurospora crassa*	ISS	UniProtKB/Swiss-Prot Q9HGR3	Kroon PA et al., 2000
FAEB_PENFN faeB: Feruloyl esterase B precursor	MAIPLVLVLAWLLPVVLAASLTQVNNFGDNP GSLQMYIYVPNKLASKPAIIVAMHPCGGS ATEYYGMYDYHSPADQYGYILIYPSATRDYN CFDAYSSASLTHNGGSDSLSIVNMVKYVI STYGADSSKVYMTGSSSGAIMTNVLAGAYP DVFAAGSAFSGMPYACLYGAGAADPIMSNQ TCSQGQIQHTGQQWAAYVHNGYPGYTGQY PRLQMWHGTADNVISYADLGQEISQWTTIM G LSFTGNQTNTPLSGYTKMVYGDGSKFQAYS AAGVGHFVPTDVSVVLDWFGITSGTTTTTT PTTTPTTSTSPSSTGGCTAAHWAQCGGIGY SGCTACASPYTCQKANDYYSQCL	*Penicillium funiculosum*	IDA	UniProtKB/Swiss-Prot Q9HE18	Kroon PA et al., 2000
FAEB_PIREQ faeB: Feruloyl esterase B precursor	MKTSIVLSIVALFLTSKASADCWSERLGWPC CSDSNAEVIYVDDDGDWGVENNDWCGIQK EEENNNSWDMGDWNQGGNQGGGMPWG DFGGNQGGGMQWGDFGGNQGGGMPWG DFGGNQGG GMPWGDFGGNQGGNQGGGMPWGDFGG NQGGNQGGGMPWGDFGGNQGGGMQWG DFGGNQGG NQGGGMPWGDFGGNQGGGMQWGDFGG NQGGNQGGGMPWGDFGGNQGGGMQWG DFGGNQGG GMQWGDFGGNQGGNQDWGNQGGNSGPT VEYSTDVDCSGKTLKSNTNLNINGRKVIVKF PS GFTGDKAAPLLINYHPIMGSASQWESGSQT AKAALNDGAIVAFMDGAQGPMGQAWNVGP C CTDADDVQFTRNFIKEITSKACVDPKRIYAAG FSMGGGMSNYAGCQLADVIAAAAPSAFD LAKEIVDGGKCKPARPFPILNFRGTQDNVVM YNGGLSQVVQGKPITFMGAKNNFKEWAKM NGCTGEPKQNTPGNNCEMYENCKGGVKV GLCTINGGGHAEGDGKMGWDFVKQFSLP	*Piromyces equi*	IDA	UniProtKB/Swiss-Prot Q9Y871	Fillingham IJ et al., 1999

*Gene Ontology Evidence Codes: ISS; Inferred from Sequence or Structural Similarity. IDA; Inferred from Direct Assay

FAEs to date involves the isolation, (Topakas et al., 2006) purification, and characterization of FAEs derived from a wide range of microorganisms (fungi and bacteria), as well as the enzymetic release of the products from cell wall degradation (Figure 4). Generally, FAE enzymes are produced (purified and characterized) from several microorganisms (various bacteria and fungi).

FERULOYL ESTERASES, RESEARCH AND INDUSTRY

Generally, feruloyl esterases benefit microorganisms, industry, and biochemists (Figure 5). There has recently been considerable interest in a large number of potential applications of feruloyl esterases due to their roles in

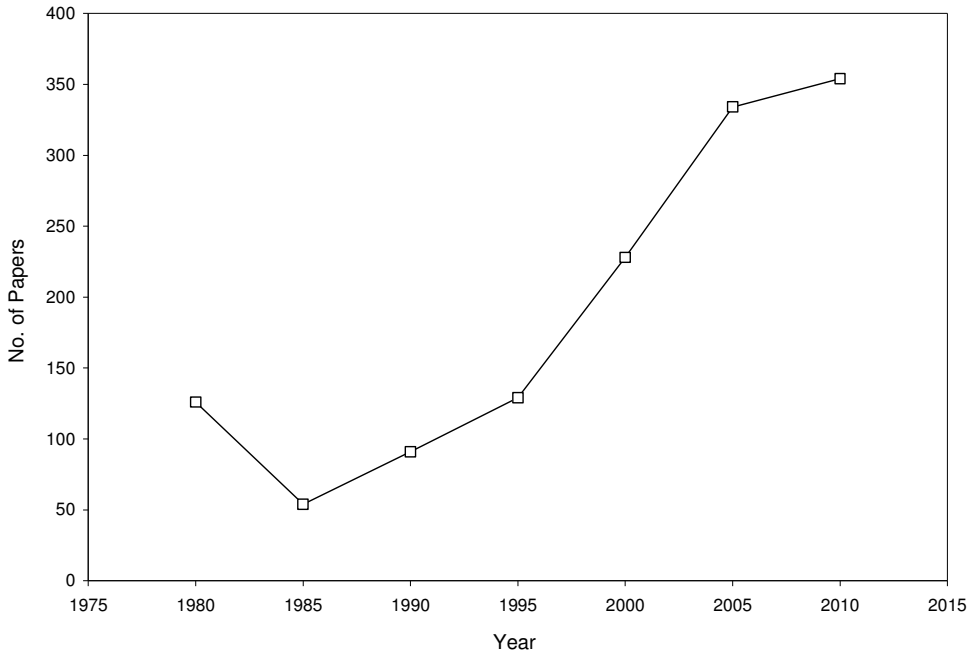


Figure 3. Annual publications on ferulic acid.

many biotechnological processes, in various industries (chemicals, fuel, animal feed, textile and laundry, pulp and paper, food and agriculture, and pharmaceutical), also in 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 FAEs has fueled much interest in these enzymes, as shown by the increasing number of FAEs discovered in microbial organisms in recent years.

FERULOYL ESTERASES APPLICATIONS IN PULP AND PAPER INDUSTRY

A great extent of work has been done during the last few decades to investigate many enzymes in pulp and paper industry. In spite of extensive research no viable technology has so far been developed to utilize the oxidative enzymes for the pulp and paper industry. The major application is the use of xylanases in pulp bleac-hing. Xylanases liberate lignin fragments by hydrolysing residual xylan. This reduces considerably the need for chlorine based chemicals for bleaching process. Other minor enzyme applications in pulp production include the use of enzymes to remove fine particles from pulp. This facilitates water removal. FAEs could be used in pulp and paper processes (Record et al. 2003; Sigoillot et al., 2005). Hemicellulases and cellulases offer alternatives to augment chemical and mechanical paper-pulping methods, and there is a large extent of published work on this subject (Kroon et al. 1996; Williamson et al., 1998). Acetylxylan esterases and FAEs might enhance this process by removing substitutions and linkages between polymers during pulping, thus making the solubilization of lignin–carbohydrate complexes easier (Mathew et al., 2004; Topakas et al., 2004a, b; Tarbouriech et al., 2001; Faulds et al., 1993; Ralet et al., 1994; Kroon et al., 1999,1996a, b; Fillingham et al., 1999; Williamson et al., 1998). Pretreatment of lignocellulosic material by secreted fungal enzymes leads to de-esterification, which increased the rate of *in vitro* digestion by ruminal microorganisms by approximately 80% (Mathew et al., 2004; Topakas et al., 2004a, b; Tarbouriech et al., 2005; Faulds et al., 1993; Ralet et al., 1994; Kroon et al., 1999, 1996a, b; Fillingham et al., 1999; Williamson et al., 1998). In paper making, enzymes are used especially for modification of starch, which is used as an important additive. Starch improves the strength, stiffness and erasability of paper. The starch suspension must have a certain viscosity, which is achieved by adding amylase enzymes in a controlled process. Enzymes used in paper industry should not contain cellulose-degrading activity as a side activity because this activity would damage the cellulose fibres (Nethaji et al., 1988; Mathew et al., 2004) to facilitate nutrient assimilation. Pitch is a sticky substance present mainly in softwoods. It is composed of lipids. It is a special problem when mechanical pulps of red pine are

Table 3. Some PDB entries of feruloyl esterases.

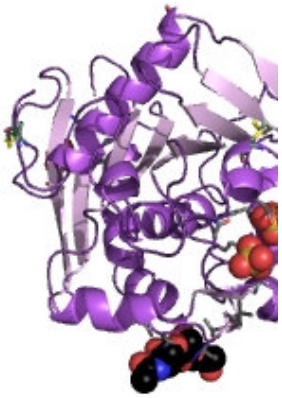
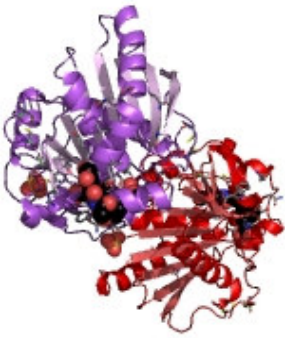
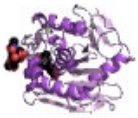
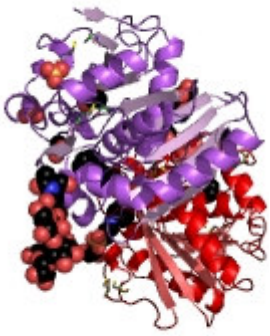
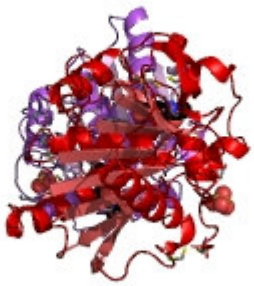
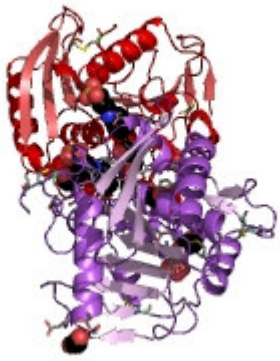
PDB Code	Title	Protein Source	Protein Structure	3D View	Reference
1usw	Crystal structure of ferulic acid esterase from aspergillus niger	Aspergillus niger. **Strain**: cbs 120.49/n400. **Expressed in**: pichia pastoris	Feruloyl esterase A. **Chain**: A. **Synonym**: ferulic acid esterase, FAE-III.		J.A.Hermoso et al. (2004)
1uwc	Feruloyl esterase a. Chain: a, b. Synonym: ferulic acid esterase a, fae-iii, cinnamoyl esterase. Engineered: yes. Other_details: n-acetylglucosamine at asn 79	Aspergillus niger. **Expressed in**: aspergillus oryzae	Feruloyl esterase a. **Chain**: a, b. **Synonym**: ferulic acid esterase a, FAE-III, cinnamoyl esterase.		K.E.McAuley et al. (2004)
2bjh	Crystal structure of s133a anfaea-ferulic acid complex	Aspergillus niger. Strain: cbs 120.49-n400. **Expressed in**: pichia pastoris	Feruloyl esterase a. **Chain**: a, b, c. **Synonym**: ferulic acid esterase a, FAE-III, cinnamoyl esterase.	  	C.B.Faulds et al. (2005)
2hl6	Structure of homologously expressed ferrulate esterase of aspergillus niger in complex with caps	Aspergillus niger. Fungi. Gene: FAE-A. **Expressed in**: aspergillus niger.	Feruloyl esterase A. **Chain**: a, b. **Synonym**: ferulic acid esterase a, FAE-III, cinnamoyl esterase, ferrulate esterase A.		I.Benoit et al. (2006)

Table 3. Continued.

1uza	Crystallographic structure of a feruloyl esterase from aspergillus niger	Aspergillus niger. **Expressed in**: aspergillus oryzae	Feruloyl esterase a. **Chain**: a, b. **Synonym**: ferulic acid esterase A, FAE-III, cinnamoyl esterase.		K.E.McAuley et al. (2004).
2ix9	Respective role of protein folding and glycosylation in the thermal stability of recombinant feruloyl esterase a	Aspergillus niger. Strain: d15pyrg. **Expressed in**: escherichia coli.	Feruloyl esterase a. **Chain**: a, b. Fragment: catalytic domain, residues 22-281. **Synonym**: feruloyl esterase types A, FAE-III, cinnamoyl esterase.		I.Benoit et al. (2006).

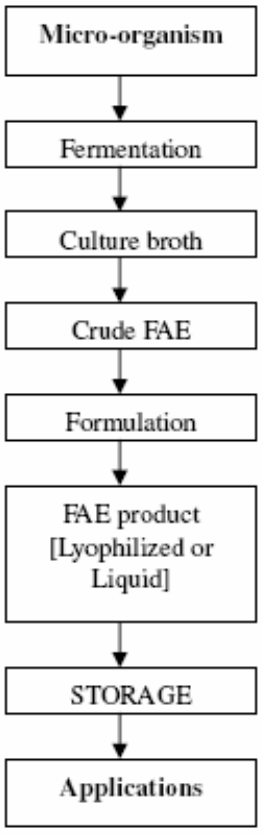


Figure 4. Flow diagram for the life cycle of feruloyl esterases from production to industry

used as a raw material. Pitch causes problems in paper machines and can be removed by lipases.

FERULOYL ESTERASES IN FOOD AND PHARMACEUTICAL INDUSTRY

FAEs derived from non-recombinant producing strains might be important for better acceptance by consumer's food related applications. In addition, ferulic acid, which is the most abundant cinnamic acid in plant cell walls, is a precursor for vanillin and its access through biotechnological methods is crucial in the quest for natural vanillin (Priefert et al., 2001). As well as being exploited as a hydrolase, FAE was shown to be a good catalyst in synthesizing sugar-phenolic esters (Mathew et al., 2004; Topakas et al., 2004a, b), and could also be used to functionalize sugar polymers by adding phenolic deriva-tives onto the natural biopolymers.

Some of the ester-linked substituents on plant cell wall polysaccharides retard or inhibit microbial infection. There are many examples in published reports concerning the antimicrobial nature of the phenolic compounds towards some microorganisms. Phenolic components of the plant cell wall, especially p-coumaric acid, ferulic acid, and p-hydroxybenzaldehyde, inhibit the growth of rumen microorganisms (Williamson et al. 1998) and phenolic acids derived from plant cell walls have long been used as food preservatives (Garcia-Conesa et al., 1998a,b) to inhibit microbial growth. *Magnaporthe grisea*, a rice blast fungus,

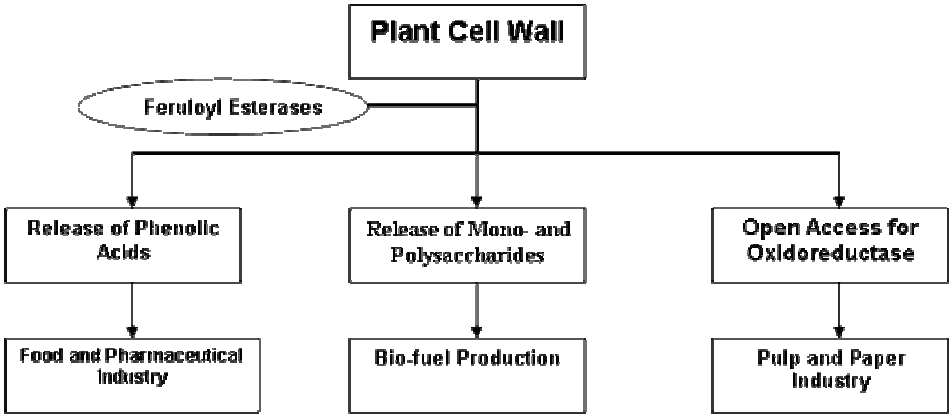


Figure 5. Diagrammatic representation of industrial and medicinal applications of feruloyl esterases

produces a xylanase and an arabinofuranosidase that act synergistically to release arabinoxylo-oligosaccharides from rice cell walls. These compounds contain esterified ferulic acid, and the release leads to the death (presumably programmed cell death) of surrounding rice cells. *In vitro* removal of the ferulic acid moiety destroyed more than 95% of this killing ability, which would enhance the chances of the colonization of the rice cell wall by the pathogen in the presence of a cinnamoyl esterase (Saija et al., 1999).

As phenolic acid sugar esters have clear antitumor activity and the potential to be used to formulate antimicrobial, antiviral, and/or anti-inflammatory agents (Omar et al., 1992; Garcia-Conesa et al., 2004), specific FAEs could be used in the tailored synthesis of such pharmaceuticals (Garcia-Conesa et al., 2004; Malherbo et al., 2002; Kondo et al., 1990). The FAE system of *T. stipitatus* has been studied and three discrete FAEs, including a type C esterase, with broad specificity against hydroxycinnamate esters (Kondo et al., 1990; Lam et al., 1990; Moore et al., 1996) have been found. The re-establishment of efficient use of cheap agricultural waste materials, with their synergistic action with other lignocellulose-degrading enzymes, is promising tools in various agro-industrial processes (Moore et al., 1996; Benoit et al., 2006). Other potential applications include production of important medicinal compounds, improvement of bread quality, pulp treatment, juice clarification, improvement of quality of animal feedstock, production of biofuel, and synthesis of oligosaccharides (Williamson et al., 1998). Therefore, effective FAEs production is a vital prerequisite for successful applications in various Industries. To achieve this goal, it is necessary to use FAEs, as well as defined polysaccharides and oligosaccharides from different agricultural raw materials.

FAEs are potential analytical aids in modern carbohydrate chemistry. In combination with other plant cell wall-degrading enzymes, the esterases will provide important tools in understanding the fine structure and linkage patterns that exist in the plant cell wall, but the science is at an early stage and ripe for exploitation (Mathew et al., 2004; Topakas et al., 2003a; Tarbouriech et al., 2005; Faulds et al., 1993; Ralet et al., 1994; Kroon et al., 1999, 1996a, b; Fillingham et al., 1999; Williamson et al., 1998). Arabinoxylans and α-glucans in the cell walls of barley have been shown to be associated either together or to a common component through an ester bond, as shown by specific hydrolysis by a pure cinnamoyl esterase (Campbell et al., 1998; Benoit et al., 2006). The exact nature of the covalent bond between lignin and carbohydrate polymers in the cell wall matrix of various plants has still to be determined, although evidence is beginning to accrue on these structures. FAEs could provide a useful tool in helping to determine this link (Campbell et al., 1998; Sun et al., 2002). FAEs are secreted by a number of bacterial and fungal organisms that exploit plants either to enter the plant cell or to use the cell wall material as a nutritional resource. The complete degradation of plant cell wall polymers requires multi-enzyme complex systems. Most FAEs have been shown to act synergistically with xylanases, cellulases, and pectinases to break down complex plant cell wall carbohydrates (Sun et al., 2002; Sahan et al., 2003; Sorensen et al., 2003).

By-products of the maize industry are ideal stock materials for biotechnology processes. An example is ferulic acid, an aromatic food anti-oxidant that can be isolated from maize fiber after wet milling and is converted to valuable compounds such as vanillin, an important flavoring agent used extensively for the foodstuffs (Luonteri et al., 1999; Sorensen et al., 2003). Apart from its use for flavoring, vanillin is also required for the synthesis of pharmaceutical drugs and is used extensi-

vely in the perfume and metal plating industries. In agriculture, it has herbicidal action, and can be used as a ripening agent to increase the yield of sucrose in sugar cane (Luonteri et al., 1999; Wood et al., 1996).

FERULOYL ESTERASES IN BIO-FUEL INDUSTRY

The demand for ethanol has been continuously increasing, where it is used either as a chemical feedstock or as an octane enhancer or petrol additive. Global crude oil production is predicted to decline from 25 billion barrels to approximately 5 billion barrels in 2050 (Benoitet et al., 2006). In the USA, fuel ethanol has been used in gasohol or oxygenated fuels since the 1980s. These gasoline fuels contain up to 10% ethanol by volume. It is estimated that 4.54 billion liters of ethanol is used by the American transportation sector and that this number will rise phenomenally as the American automobile manufacturers plan to manufacture a significant number of flexi-fueled engines which can use a blend of 85% ethanol and 15% gasoline by volume. The production of fuel ethanol from sugars or starch impacts negatively on the economics of the process, thus making ethanol more expensive compared with fossil fuels. Hence the technology development focus in the production of ethanol has shifted towards the use of residual lignocellulosic materials to lower production cost. An improved use of wheat endosperm by-products in this type of ethanol production would generate a fermentable hydrolysate based on the hemicellulose fraction. Complete enzymatic hydrolysis of arabinoxylan requires both depolymerizing and side-group cleaving enzyme activities such as FAEs. Any hemicellulose containing lignocellulose generates a mixture of sugars upon pretreatment alone or in combination with enzymatic hydrolysis. In Europe, potable alcohol manufacturing plants are based on wheat endosperm processing, with the hemicellulosic by-product remaining after fermentation consisting of approximately 66% (W/W) arabinoxylan (Benoit et al., 2006). Fermentable sugars from cellulose and hemicellulose will essentially be glucose and xylose, which can be released from lingocellulosics by single or two-stage hydrolysis, thereby leading to mixtures of glucose and xylose or separate glucose- and xyloserich streams. Conventional methods, applied for bioconversion of cellulose and hemicellulose to ethanol, involve acid or enzyme hydrolysis of biopolymers to soluble oligosaccharides followed by fermentation to ethanol. A synergistic action between cellulases, FAEs and xylanases may prove to be more effective when applied in a critical concentration in the saccharification of steam-exploded wheat straw (Borneman et al., 1993; Kennedy et al., 1999).

CONCLUSION

Feruloyl esterases are promising enzymes to have potential and significant contributions in research and industry. However, two of the problems to commercialize the use of feruloyl esterases are: to date there is no commercial full purified feruloyl esterases and the lack of sufficient enzyme stocks. Thus, efforts have to be made in order to achieve cheap overproduction of feruloyl esterases in heterologous hosts and also their modifica-tion by chemical means or protein engineering to obtain more robust and active purified feruloyl esterases enzymes.

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