Appl Microbiol Biotechnol (2004) 63: 647–652
DOI 10.1007/s00253-003-1476-3

MINI-REVIEW

V. F. Crepin · C. B. Faulds · I. F. Connerton

Functional classification of the microbial feruloyl esterases

Received: 3 September 2003 / Revised: 23 September 2003 / Accepted: 26 September 2003 / Published online: 6 December 2003

Abstract Feruloyl esterases have potential uses over a broad range of applications in the agri-food industries. In recent years, the number of microbial feruloyl esterase activities reported has increased and, in parallel, even more related protein sequences may be discerned in the growing genome databases. Based on substrate utilisation data and supported by primary sequence identity, four sub-classes have been characterised and termed type-A, B, C and D. The proposed sub-classification scheme is discussed in terms of the evolutionary relationships existing between carbohydrate esterases.

Introduction

Feruloyl esterases

Feruloyl esterases [E.C. 3.1.1.73; also known as ferulic acid esterases (FAE), cinnamoyl esterases, cinnamic acid hydrolases] are a subclass of the carboxylic acid esterases (E.C. 3.1.1.1) that are able to hydrolyse the ester bond between hydroxycinnamic acids and sugars present in the plant cell walls (Williamson et al. 1998b). These enzymes have previously been classified as type A (e.g. *Aspergillus niger* FaeA) and type B (e.g. *A. niger* CinnAE, FaeB, *Penicillium funiculosum* FaeB, *Neurospora crassa* Fae-1), based on their specificity for the substrate aromatic moiety, specificity for the linkage to the primary sugar and ability to release ferulic acid dehydrodimers from esterified substrates (Crepin et al. 2003a; De Vries et al. 2002b; Kroon et al. 1999, 2000). The substrate utilisation of either methyl sinapate or methyl caffeate by feruloyl esterases is the basis of this classification. In addition, the majority of these esterases show acetyl xylan esterase activity, regardless of whether they are assigned as acetyl or feruloyl esterases. However, specific acetyl xylan esterase activity is rather low for the enzymes classified as feruloyl esterases. The two major feruloyl esterases of *A. niger* have received most attention, namely AnFaeA and AnFaeB (De Vries et al. 1997, 2002b; Faulds and Williamson 1994; Kroon et al. 1996, 1997).

V. F. Crepin · I. F. Connerton (✉)
Division of Food Sciences, School of Biosciences, University of Nottingham,
Sutton Bonington Campus,
Loughborough, LE12 5RD, UK
e-mail: ian.connerton@nottingham.ac.uk
Tel.: +44-115-9516161
Fax: +44-115-9516162

C. B. Faulds
Food Materials Science Division, Institute of Food Research,
Norwich Research Park, Colney,
Norwich, NR4 7UA, UK

Type A feruloyl esterases

Type A feruloyl esterases tend to be induced during growth on cereal-derived substrates. The enzyme releases ferulic acid from 1,5-ester-linked feruloylated arabinose (Ralet et al. 1994) and is also able to release low quantities of 5,5′- and 8-*O*-4′-ferulate dehydrodimers from plant material when pre-treated with a xylanase or when a xylanase is co-incubated with the feruloyl esterase (Bartolome et al.1997; Faulds and Williamson 1995; Kroon et al. 1999; Williamson et al. 1998a). There is no report of these enzymes releasing diferulate from pectin-derived material. Type A feruloyl esterases also show a preference for the phenolic moiety of the substrate that contains methoxy substitutions, especially at carbon(s) 3 and/or 5, as occurs in ferulic and sinapic acids. Regarding specificity against synthetic substrates, type A feruloyl esterases are active against methyl ferulate, methyl sinapate and methyl *p*-coumarate, but not methyl caffeate. They appear to prefer hydrophobic substrates with bulky substituents on the benzene ring (Kroon et al. 1997, 1999; Williamson et al. 1998a).

Type B feruloyl esterases

Type B feruloyl esterases are preferentially secreted by growth on sugar beet pulp. The enzymes release ferulic acid ester-linked to either C-2 of feruloylated arabinose or C-6 of feruloylated galactose residues (Kroon and Williamson 1996), but they are unable to release the dimeric forms of ferulate. The *A. niger* type B enzyme also works on 1,5-linked ferulic acid as found in cereals but at a lower rate than the type A esterases (Kroon et al. 1996; Ralet et al. 1994; Williamson et al. 1998a). The type B feruloyl esterases show a preference for the phenolic moiety of the substrate that contains one or two hydroxyl substitutions, as found in *p*-coumaric and caffeic acids, respectively. Hydrolytic rates are significantly lowered when a methoxy group is present. Regarding specificity against synthetic substrates, type B feruloyl esterases are active against methyl caffeate, methyl ferulate and methyl *p*-coumarate, but not methyl sinapate.

A third type of esterase

A third type of esterase with broad specificity against synthetic hydroxycinnamic acids (ferulic, caffeic, *p*-coumaric, sinapic) has been reported as an acetyl esterase (XYLD) from *Pseudomonas fluorescens* subsp. *cellulosa* (Ferreira et al. 1993). The data indicate that XYLD is able to cleave ester linkages in plant cell walls, although the enzyme exhibits greatest activity towards the release of acetic acid from 4-nitrophenyl acetate. The hydrolysis rate of XYLD esterase, both for hydroxycinnamic acids and for acetylated substrates, is significantly lower (25- to 500-fold) than reported for the characterised fungal feruloyl esterases (De Vries et al. 2002b; Faulds and Williamson 1994; Kroon et al. 1996, 2000) and other microbial acetyl xylan esterases (Basaran and Hang 2000; Blum et al. 1999; Degrassi et al. 2000; Linden et al. 1994; McDermid et al. 1990). In addition, XYLD is capable of releasing 5-5′-ferulic dehydrodimers from barley and wheat cell walls (Bartolome et al. 1997). In respect of this broad substrate specificity, XYLD has been tentatively classified as a general plant cell wall esterase.

Feruloyl esterases are subject to complex regulation

The expression of many fungal genes coding for cell wall-degrading enzymes are subject to complex regulation through carbon catabolite repression, as exercised by the repressor CreA from *A. niger* and *A. nidulans* (De Vries et al. 1999, 2002a; Dowzer and Kelly 1991; Orejas et al. 2001; Ruijter and Visser 1997), Cre-1 from *N. crassa* (Ebbole 1998), or Cre1 from *Trichoderma reesei* (Strauss et al. 1995). Cell wall-degrading enzymes of *A. niger* are also specifically induced via the xylanolytic transcriptional activator XlnR (De Vries and Visser 1999; van Peij et al. 1998), in addition to positive or negative regulation by factors derived from the growth substrates themselves (De Vries et al. 2002b; Faulds and Williamson 1999; Faulds et al. 1997). Feruloyl esterase activity is proposed to be regulated through the xylose-induced expression of the repressor protein XlnR in *Aspergillus* during growth on hemicellulosic material (De Vries and Visser 2001), but it has yet to be established how type B esterases are induced during growth on material such as sugar beet pulp.

Protein sequence databases

In recent years, the number of microbial feruloyl esterase activities reported has increased, in particular with the acquisition of related protein sequences in the growing genome databases. Several enzymes have been purified and characterised from aerobic and anaerobic micro-organisms that utilise plant cell wall carbohydrates (Blum et al. 2000; Borneman et al. 1992; Castanares et al. 1992; Crepin et al. 2003a; Dalrymple et al. 1996; De Vries et al. 2002b; Donaghy et al. 2000; Faulds and Williamson 1991, 1994; Ferreira et al. 1993; Fillingham et al. 1999; Kroon et al. 1996, 2000; McCrae et al. 1994; Tenkanen et al. 1991). Feruloyl esterase activities have also been detected in mammalian cells and plants (Andreasen et al. 2001; Sancho et al. 1999) but these enzymes have yet to be purified and their protein sequences determined for comparison with the microbial enzymes. However, a number of microbial gene sequences have been determined; and the predicted protein sequences from these suggests there is a considerable degree of structural diversity between them. Analysis of predicted feruloyl esterase protein sequences show that many of these enzymes are modular, comprising of a catalytic domain covalently fused to non-catalytic carbohydrate-binding modules (Ferreira et al. 1993; Kroon et al. 2000). Alternatively, the catalytic domain may be included in a greater modular complex, such as that observed for the cellulosome of *Clostridium* sp. (Blum et al. 2000; Shoham et al. 1999) or for the multiprotein cellulose-binding cellulase-hemicellulase complex from the anaerobic fungus *Piromyces equi* (Fillingham et al. 1999). Although the first crystal structure of a feruloyl esterase module was reported recently for two esterases from *C. thermocellum* (Prates et al. 2001; Schubot et al. 2001), relatively few studies have been performed to elucidate the functional relationships between sequence-diverse feruloyl esterases. At present, the lack of highly conserved sequences within the sequenced esterases does not permit further classification for the feruloyl esterases, other than that their primary amino acid sequences place them in family 1 (Coutinho and Henrissat 1999) of the carbohydrate esterase classification (http://afmb.cnrs-mrs.fr/~cazy/CAZY/).

Based on the primary sequence identity and activity profile data reported for more than 20 feruloyl esterases against synthetic methyl esters, we propose a scheme for the sub-classification of the feruloyl esterase enzymes. The classification is discussed in terms of the evolutionary relationships existing between carbohydrate esterases.

Materials and methods

Phylogenetic analyses

Multiple alignments of sequences or domains showing feruloyl esterase activity and sequence-related enzymes, such as lipases, and the construction of a neighbour-joining phylogenetic tree (Saitou and Nei 1987) were performed with the CLUSTAL W program, available at http://www.ebi.ac.uk/clustalw/ (Thompson et al. 1994). The matrix used for the multiple sequence alignment was the group of BLOSUM matrices recommended for amino acid sequences alignments (Henikoff and Henikoff 1992). The gap penalties were set at the default values, the gap separation penalty value was 8, the penalty value for extending a gap was 0.05 and the penalty value for opening a gap was 10.

Results and discussion

Feruloyl esterase sub-classification

Using literature reports of the substrate preferences of characterised feruloyl esterases, together with the growth substrate requirements for crude cell wall materials leading to the expression of these enzymes by various microbes, we organised the microbial feruloyl esterases into four functional classes. Using the published compatible data on the feruloyl esterases *Pen. funiculosum* FaeB (EMBL accession number AJ291496), *N. crassa* Fae-1 (AJ293029), *Ps. fluorescens* XYLD (X58956), *Pen. funiculosum* FaeA (AJ312296), *Piromyces equi* EstA (AF164516), *A. niger* FaeA (AF361950); *Talaromyces stipitatus* FaeC (AJ505939) and *A. niger* FaeB (AJ309807), a comparative analysis indicated these enzymes could be divided into four putative sub-classes, termed types A, B, C and D (Table 1). Members of each sub-class show similar activity profiles against four model substrates [methyl 3-methoxy-4-hydroxycinnamate (MFA), methyl 3,4-dihydroxycinnamate (MCA), methyl 3,5-dimethoxy-4-hydroxycinnamate (MSA), methyl 4-hydroxycinnamate (M*p*CA)] and similar abilities to release diferulate or not from plant cell wall material. Moreover, these classes are likely to reflect the substrate availability of natural plant cell walls, as they are preferentially induced by their respective organisms when grown in the presence of similar substrates. Finally, it can be demonstrated that members of the same activity class also share a high degree of amino acid sequence identity.

In order to investigate further the feruloyl esterase sub-classification, a phylogenetic tree was drawn on the basis of primary sequence identity between selected plant cell wall-acting esterases and other sequence related enzymes, such as lipases and xylanases. A phylogenetic tree of the cladogram type was constructed by the neighbour-joining method, using CLUSTAL W. A cladogram is a branching tree assumed to be an estimate of a phylogeny where the branches are of equal length, which shows common ancestry but does not indicate the degree of evolutionary time separating the taxa. The phylogenic tree indicates that feruloyl esterases diverged in three main branches, within which they evolved along discrete lines to form sub-classes of specialised enzymes (Fig. 1).

The phylogenetic analysis by and large supports the feruloyl esterase classification proposed above on the basis of enzyme activity profiles against synthetic methyl esters. However, it is of note that the phylogenetic analysis indicates that TsFaeC (EMBL accession number AJ505939) and AnFaeB (AJ309807) are closely related and evolved from a common root. As a consequence, we suggest that these enzymes belong to the same sub-class of feruloyl esterases, termed type C, despite the reported

Table 1 Classification of feruloyl esterases based on primary amino acid sequence identity, specificity for hydroxycinnamic acid methyl esters, ability to release 5,5′-diferulic acid from model and complex substrates and inducible plant cell wall materials. *CE* carbohydrate esterase; for other abbreviations, see Materials and methods

Parameter	Type A	Type B	Type C	Type D
	Aspergillus niger FaeA[a,c,d]	*Penicillium funiculosum* FaeB[e], *Neurospora crassa* Fae-1[f]	*A. niger* FaeB[b,c,d], *Talaromyces stipitatus* FaeC[g]	*Pen. equi* EstA[h], *Pseudomonas fluorescens* XYLD[i]
Preferential induction medium	WB	SBP	SBP–WB	WB
Hydrolysis of methyl esters	MFA, MSA, M*p*CA	MFA, M*p*CA, MCA	MFA, MSA, M*p*CA, MCA	MFA, MSA, M*p*CA, MCA
Release of diferulic acid	Yes (5-5′)	No	No	Yes (5-5′)
Sequence similarity	Lipase	CE family 1 acetyl xylan esterase	Chlorogenate esterase tannase	Xylanase

[a] Faulds and Williamson (1994)
[b] Kroon et al. (1996)
[c] Kroon et al. (1999)
[d] Ralet et al. (1994)
[e] Kroon et al. (2000)
[f] Crepin et al. (2003a)
[g] Crepin et al. (2003b)
[h] Fillingham et al. (1999)
[i] Ferreira et al. (1993)

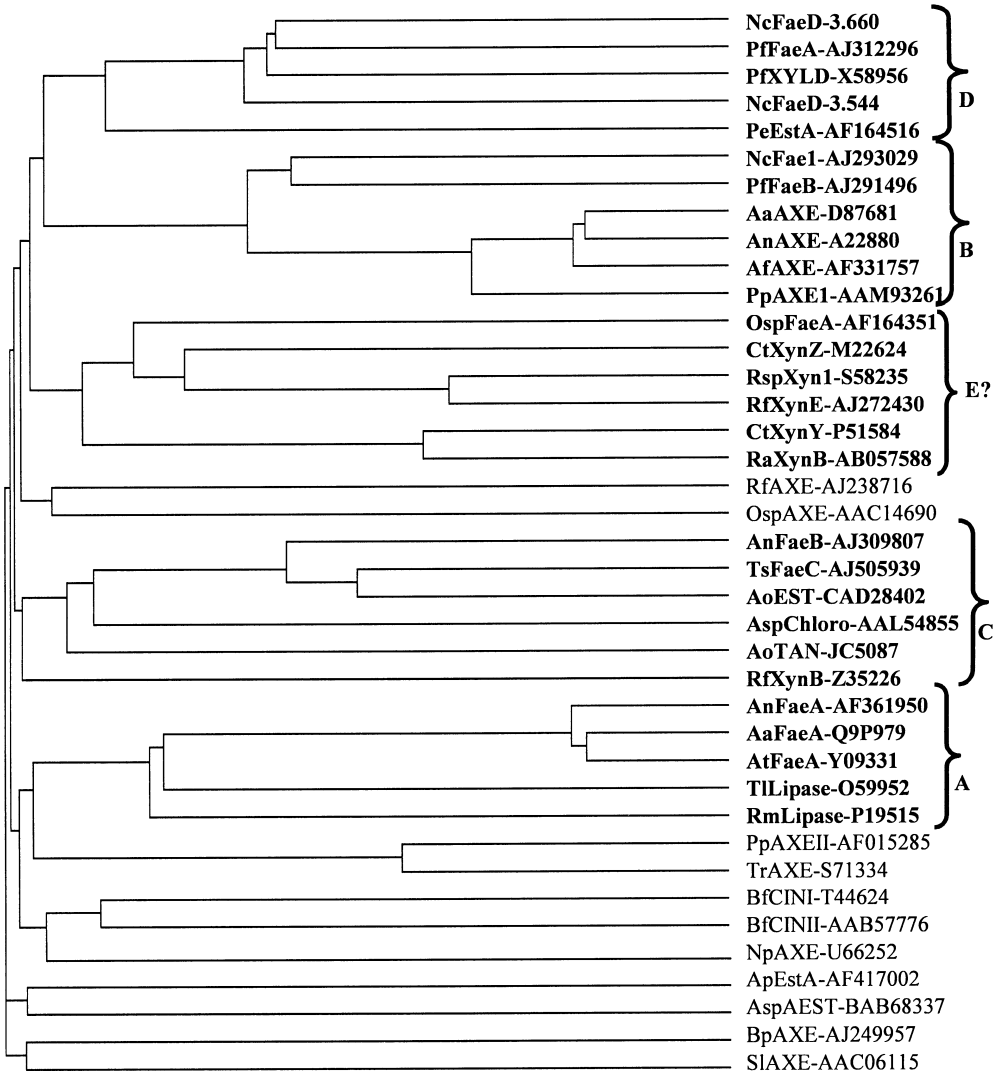

Fig. 1 Cladogram of enzymes of the feruloyl esterase family and relatives. Sequence names with their accession numbers are *shown on the right of the tree*. *NcFaeD-3.660 Neurospora crassa* FaeD-3.660 feruloyl esterase, *PfFaeA Penicillium funiculosum* FaeA (AJ312296), *PfXYLD Pseudomonas fluorescens* XYLD (X58956), *NcFaeD-3.544 N. crassa* FaeD-3.544 feruloyl esterase (this work), *PeESTA Piromyces equi* ESTA (AF164516), *NcFae-1 N. crassa* Fae-1 (AJ293029), *PfFaeB Pen. funiculosum* FaeB (AJ291496), *AaAXE Aspergillus awamori* AXE (D87681), *AnAXE Asp. niger* AXE (A22880), *AfAXE Asp. ficuum* AXE (AF331757), *PpAXEI Pen. purpurogenum* AXE I (AAM93261), *OspFaeA Orpinomyces* sp. PC-2 FaeA (AF164351), *CtXynZ Clostridium thermocellum* XynZ (M22624), *RspXyn1 Ruminococcus* sp. Xyn1 (S58235), *RfXynE R. flavefaciens* XynE (AJ272430), *CtXynY C. thermocellum* XynY (X83269), *RaXynB R. albus* XynB (AB057588), *RfAXE R. flavefaciens* AXE (AJ238716), *OspAXE Orpinomyces* sp. PC-2 AXE A (AAC14690), *AnFaeB Asp. niger* FaeB (AJ309807), *TsFaeC Talaromyces stipitatus* FaeC (AJ505939), *AoEST Asp. oryzae* selective esterase (CAD28402), *AspChloro Acinetobacter* sp. chlorogenate esterase (AAL54855), *AoTAN Asp. oryzae* tannase (JC5087), *RfXynB R. flavefaciens* XynB (Z35226), *AnFaeA Asp. niger* FaeA (AF361950), *AaFaeA Asp. awamori* FaeA (Q9P979), *AtFaeA Asp. turbingensis* FaeA (Y09331), *TlLipase Thermomyces lanuginosus* lipase (O59952), *RmLipase Rhizomucor miehei* lipase (P19515), *PpAXE P. purporogenum* xylan esterase II (AF015285), *TrAXE T. reesei* acetyl xylan esterase I (S71334), *BfCINI Butyrivibrio fibrisolvens* E14 cinnamoyl ester hydrolase I (T44624), *BfCINII B. fibrisolvens* E14 cinnamoyl ester hydrolase II (AAB57776), *NpAXE Neocallimastix patriciarum* AXE (U66253), *ApESTA Asp. parasiticus* ESTA esterase (AF417002), *AspAEST Acinetobacter* sp. AEST esterase (BAB68337), *BpAXE Bacillus pumilus* AXE (AJ249957), *SlAXE Streptomyces lividans* AXE (AAC06115)

difference in the substrate specificity of AnFaeB for methyl hydroxycinnamates, which initially suggested this enzyme to be a type B feruloyl esterase (De Vries et al. 2002b). The true type B enzymes have a common node, but the protein sequences of *Pen. funiculosum* FaeB (AJ291496) and *N. crassa* Fae-1 (AJ293029) may be distinguished from the high degree of conservation observed between the acetyl xylan esterases of *A. awamori* (D87681), *A. niger* (A22880), *A. ficuum* (AF331757) and *Pen. purpurogenum* (AAM93261).

The phylogenetic tree also recognises sequence relationships outside the present scheme. For example, the acetyl xylan esterase II from *Pen. purpurogenum* (Accession number AF015285) and the catalytic domain of AXE I (acetyl xylan esterase) from *Trichoderma reesei* (S71334) share 67% sequence identity (Gutierrez et al. 1998); and

enzymes such as XynY and XynZ from *C. thermocellum* (Accession numbers P51584 and M22624, respectively), FaeA from *Orpinomyces* sp. PC-2 (AF164351) and Xyn1 from *Ruminococcus* sp. (S58235) are closely related (Blum et al. 2000). In parallel, the phylogenetic tree shows that, although the feruloyl esterases have some common roots, in some cases they may be more sequence-related to a variety of other enzymes, such as lipases, acetyl xylan esterases, or xylanases, than they are to each other.

Although the four enzyme sub-classes could be ascribed based on functional criteria outlined above, the phylogenetic analysis also suggests that there are probably yet further unnamed enzyme sub-classes. This fifth sub-class is comprised of OspFaeA (AF164351), CtXynZ (M22624), RspXyn1 (S58235), RfXynE (AJ272430), CtXynY (P51584) and RaXynB (AB057588). However, at present, this grouping may only be discriminated on the basis of their primary amino acid sequence identity, as no further correlation could be established here due to the lack of comparable enzyme activity data and, in some cases, information as to which plant cell wall materials preferentially provoke the expression of these feruloyl esterases by the microbes that possess them. Future acquisition of protein and predicted protein sequences with complementary enzyme activity data using synthetic methyl esters will enable the substantiation of these and potentially other enzymes classes recognised in phylogenetic analyses. These data will in turn provide a better understanding of the complex evolutionary relationship between the feruloyl esterases.

In conclusion, we propose that feruloyl esterases be classified into four functional sub-classes, termed types A, B, C and D, based on similarities in esterase activity profiles against synthetic methyl esters and underpinned by the intra- and inter-group protein sequence identities of the enzymes.

Acknowledgements We would like to thank the Biotechnology and Biological Sciences Research Council (BBSRC), UK, and the Department of Trade and Industry, UK, for their support through the LINK Applied Biocatalysts programme (Grant number BTL/27/12).

References

Andreasen MF, Kroon P, Williamson G, Garcia-Conesa MT (2001) Esterase activity able to hydrolyze dietary antioxidant hydroxycinnamates is distributed along the intestine of mammals. J Agric Food Chem 49:5679–5684

Bartolome B, Faulds CB, Kroon PA, Waldron K, Gilbert HJ, Hazlewood G, Williamson G (1997) An *Aspergillus niger* esterase (ferulic acid esterase III) and a recombinant *Pseudomonas fluorescens* subsp. *cellulosa* esterase (XylD) release a 5-5′-ferulic dehydrodimer (diferulic acid) from barley and wheat cell walls. Appl Environ Microbiol 63:208–212

Basaran P, Hang YD (2000) Purification and characterization of acetyl esterase from *Candida guilliermondii*. Lett Appl Microbiol 30:167–171

Blum DL, Li XL, Chen H, Ljungdahl LG (1999) Characterization of an acetyl xylan esterase from the anaerobic fungus *Orpinomyces* sp. strain PC-2. Appl Environ Microbiol 65:3990–3995

Blum DL, Kataeva IA, Li XL, Ljungdahl LG (2000) Feruloyl esterase activity of the *Clostridium thermocellum* cellulosome can be attributed to previously unknown domains of XynY and XynZ. J Bacteriol 182:1346–1351

Borneman WS, Ljungdahl LG, Hartley RD, Akin DE (1992) Purification and partial characterization of two feruloyl esterases from the anaerobic fungus *Neocallimastix* strain MC-2. Appl Environ Microbiol 58:3762–3766

Castanares A, McCrae SI, Wood TM (1992) Purification and properties of a feruloyl/*p*-coumaroyl esterase from the fungus *Penicillium pinophilum*. Enzyme Microb Technol 14:875–884

Coutinho PM, Henrissat B (1999) Carbohydrate-active enzymes: an integrated database approach. In: Gilbert HJ, Davies GJ, Svensson B, Henrissat B (eds), Recent advances in carbohydrate engineering. Royal Society of Chemistry, Cambridge, pp 3–12

Crepin VF, Faulds CB, Connerton IF (2003a) A non-modular type-B feruloyl esterase from *Neurospora crassa* exhibits concentration dependent substrate inhibition. Biochem J 370:417–427

Crepin VF, Faulds CB, Connerton IF (2003b) Production and characterisation of the *Talaromyces stipitatus* feruloyl esterase FAEC in *Pichia pastoris*: identification of the nucleophilic serine. Protein Express Purif 29:176–184

Dalrymple BP, Swadling Y, Cybinski DH, Xue GP (1996) Cloning of a gene encoding cinnamoyl ester hydrolase from the ruminal bacterium *Butyrivibrio fibrisolvens* E14 by a novel method. FEMS Microbiol Lett 143:115–120

De Vries RP, Visser J (1999) Regulation of the feruloyl esterase (*faeA*) gene from *Aspergillus niger*. Appl Environ Microbiol 65:5500–5503

De Vries RP, Visser J (2001) *Aspergillus* enzymes involved in degradation of plant cell wall polysaccharides. Microbiol Mol Biol Rev 65:497–522

De Vries RP, Michelsen B, Poulsen CH, Kroon PA, Heuvel RH van den, Faulds CB, Williamson G, Hombergh JP van den, 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 63:4638–4644

De Vries RP, Visser J, Graaff LH de (1999) CreA modulates the XlnR-induced expression on xylose of *Aspergillus niger* genes involved in xylan degradation. Res Microbiol 150:281–285

De Vries RP, Jansen J, Aguilar G, Parenicova l, Joosten V, Wulfert F, Benen JAE, Visser J (2002a) Expression profiling of pectinolytic genes from *Aspergillus niger*. FEBS Lett 530:41–47

De Vries RP, Kuyk PA van, Kester HC, Visser J (2002b) 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 363:377–386

Degrassi G, Kojic M, Ljubijankic G, Venturi V (2000) The acetyl xylan esterase of *Bacillus pumilus* belongs to a family of esterases with broad substrate specificity. Microbiology 146:1585–1591

Donaghy JA, Bronnenmeier K, Soto-Kelly PF, McKay AM (2000) Purification and characterization of an extracellular feruloyl esterase from the thermophilic anaerobe *Clostridium stercorarium*. J Appl Microbiol 88:458–466

Dowzer CEA, Kelly JM (1991) Analysis of the *creA* gene, a regulator of carbon catabolite repression in *Aspergillus nidulans*. Mol Cell Biol 11:5701–5709

Ebbole DJ (1998) Carbon catabolic repression of gene expression and conidiation in *Neurospora crassa*. Fungal Genet Biol 25:15–21

Faulds CB, Williamson G (1991) The purification and characterization of 4-hydroxy-3-methoxycinnamic (ferulic) acid esterase from *Streptomyces olivochromogenes*. J Gen Microbiol 137:2339–2345

Faulds CB, 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 140:779–787

Faulds CB, Williamson G (1995) Release of ferulic acid from wheat bran by a ferulic acid esterase (FAE-III) from *Aspergillus niger*. Appl Microbiol Biotechnol 43:1082–1087

Faulds CB, Williamson G (1999) Effect of hydroxycinnamates and benzoates on the production of feruloyl esterases by *Aspergillus niger*. J Sci Food Agric 79:450–452

Faulds CB, De Vries RP, Kroon PA, Visser J, Williamson G (1997) Influence of ferulic acid on the production of feruloyl esterases by *Aspergillus niger*. FEMS Microbiol Lett 157:239–244

Ferreira LMA, Wood TM, Williamson G, Faulds C, Hazlewood GP, Black GW, Gilbert HJ (1993) A modular esterase from *Pseudomonas fluorescens* subsp. *cellulosa* contains a non-catalytic cellulose-binding domain. Biochem J 294:349–355

Fillingham IJ, Kroon PA, Williamson G, Gilbert HJ, Hazlewood GP (1999) A modular cinnamoyl ester hydrolase from the anaerobic fungus *Piromyces equi* acts synergistically with xylanase and is part of a multiprotein cellulose-binding cellulase-hemicellulase complex. Biochem J 343:215–224

Gutierrez R, Cederlund E, Hjelmqvist L, Peirano A, Herrera F, Ghosh D, Duax W, Jornvall H, Eyzaguirre J (1998) Acetyl xylan esterase II from *Penicillium purpurogenum* is similar to an esterase from *Trichoderma reesei* but lacks a cellulose binding domain. FEBS Lett 423:35–38

Henikoff S, Henikoff J (1992) Amino acid substitution matrices from protein blocks. Proc Natl Acad Sci USA 89:10915–10919

Kroon PA, Williamson G (1996) Release of ferulic acid from sugar-beet pulp by using arabinanase, arabinofuranosidase and an esterase from *Aspergillus niger*. Biotechnol Appl Biochem 23:263–267

Kroon PA, Faulds CB, Williamson G (1996) Purification and characterization of a novel esterase induced by growth of *Aspergillus niger* on sugar-beet pulp. Biotechnol Appl Biochem 23:255–262

Kroon PA, Faulds CB, Brezillon C, Williamson G (1997) Methyl phenylalkanoates as substrates to probe the active site of esterases. Eur J Biochem 248:245–251

Kroon PA, Garcia-Conesa MT, Fillingham IJ, Hazlewood GP, Williamson G (1999) Release of ferulic acid dehydrodimers from plant cell walls by feruloyl esterases. J Sci Food Agric 79:428–434

Kroon PA, Williamson G, Fish NM, Archer DB, Belshaw NJ (2000) A modular esterase from *Penicillium funiculosum* which releases ferulic acid from plant cell walls and binds crystalline cellulose contains a carbohydrate binding module. Eur J Biochem 267:6740–6752

Linden J, Samara M, Decker S, Johnson E, Boyer M, Pecs M, Adney W, Himmel M (1994) Purification and characterization of an acetyl esterase from *Aspergillus niger*. Appl Biochem Biotechnol 383:45–46

McCrae SI, Leith KM, Gordon AH, Wood TM (1994) Xylan-degrading enzyme system produced by the fungus *Aspergillus awamori*: isolation and characterization of a feruloyl esterase and a *p*-coumaroyl esterase. Enzyme Microb Technol 16:826–834

McDermid KP, Forsberg CW, MacKenzie CR (1990) Purification and properties of an acetyl xylan esterase from *Fibrobacter succinogenes* S85. Appl Environ Microbiol 56:3805–3810

Orejas M, MacCabe AP, Perez-Gonzalez JA, Kumar S, Ramon D (2001) The wide-domain carbon catabolite repressor CreA indirectly controls expression of the *Aspergillus nidulans xlnB* gene, encoding the acidic endo-β-(1,4)-xylanase X_{24}. J Bacteriol 183:1517–1523

Peij NNME van, Visser J, Graaff LH de (1998) Isolation and analysis of *xlnR*, encoding a transcriptional activator co-ordinating xylanolytic expression in *Aspergillus niger*. Microbiology 27:131–142

Prates JAM, Tarbouriech N, Charnock SJ, Fontes CMGA, Ferreira LMA, Davies GJ (2001) The structure of the feruloyl esterase module of xylanase 10B from *Clostridium thermocellum* provides insights into substrate recognition. Structure 9:1183–1190

Ralet MC, Faulds CB, Williamson G, Thibault, JF (1994) Degradation of feruloylated oligosaccharides from sugar-beet pulp and wheat bran by ferulic acid esterases from *Aspergillus niger*. Carbohydr Res 263:257–269

Ruijter GJG, Visser J (1997) Carbon repression in *Aspergilli*. FEMS Microbiol Lett 151:103–114

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425

Sancho AI, Faulds CB, Bartolomé B, Williamson G (1999) Characterisation of feruloyl esterase activity in barley. J Sci Food Agric 79:447–449

Schubot FD, Kataeva IA, Blum DL, Shah AC, Ljungdahl LG, Rose JP, Wang BC (2001) Structural basis for the substrate specificity of the feruloyl esterase domain of the cellulosomal xylanase Z from *Clostridium thermocellum*. Biochemistry 40:12524–12532

Shoham Y, Lamed R, Bayer EA (1999) The cellulosome concept as an efficient microbial strategy for the degradation of insoluble polysaccharides. Trends Microbiol 7:275–281

Strauss J, Mach RL, Zeilinger S, Hartler G, Stoffler G, Wolschek M, Kubicek CP (1995) Cre1, the carbon catabolic repressor protein from *Trichoderma reesei*. FEBS Lett 376:103–107

Tenkanen M, Schuseil J, Puls J, Poutanen K (1991) Production, purification and characterization of an esterase liberating phenolic-acids from lignocellulosics. J Biotechnol 18:69–84

Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–4680

Williamson G, Faulds CB, Kroon PA (1998a) Specificity of ferulic acid (feruloyl) esterases. Biochem Soc Trans 26:205–209

Williamson G, Kroon PA, Faulds CB (1998b) Hairy plant polysaccharides: a close shave with microbial esterases. Microbiology 144:2011–2023