Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Biotechnology 123 (2006) 413–433
Journal of BIOTECHNOLOGY
www.elsevier.com/locate/jbiotec

Review

The xylanolytic enzyme system from the genus *Penicillium*

Renato Chávez [a], Paulina Bull [b], Jaime Eyzaguirre [b,c,*]

[a] *Unidad de Biotecnología, Instituto de Investigaciones Agropecuarias (INIA), CRI-Carillanca, Temuco, Chile*
[b] *Laboratorio de Bioquímica, Departamento de Genética Molecular y Microbiología, Pontificia Universidad Católica de Chile, Santiago, Chile*
[c] *Departamento de Ciencias Biológicas, Universidad Andrés Bello, Santiago, Chile*

Received 23 July 2005; received in revised form 6 December 2005; accepted 23 December 2005

Abstract

In nature, there are numerous microorganisms that efficiently degrade xylan, a major component of lignocellulose. In particular, filamentous fungi have demonstrated a great capability for secreting a wide range of xylanases, being the genus *Aspergillus* and *Trichoderma* the most extensively studied and reviewed among the xylan-producing fungi. However, an important amount of information about the production and genetics of xylanases from fungi of the genus *Penicillium* has accumulated in recent years. A great number of *Penicillia* are active producers of xylanolytic enzymes, and the use of xylanases from these species has acquired growing importance in biotechnological applications. This review summarizes our current knowledge about the properties, genetics, expression and biotechnological potential of xylanases from the genus *Penicillium*.

Keywords: *Penicillium*; Xylanases; Gene expression; Biotechnological applications

Contents

* Corresponding author. Tel.: +56 2 6618070.
E-mail address: jeyzag@bio.puc.cl (J. Eyzaguirre).

0168-1656/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jbiotec.2005.12.036

1. Introduction

Xylan is the principal component of plant cell wall hemicelluloses. It is a heteroglycan composed of a linear chain of xylopyranose residues bound by $\beta\,(1 \rightarrow 4)$ linkages, with a variety of substituents linked to the main chain by glycosidic or ester linkages (Joseleau et al., 1992). These substituents are mainly acetate, methyl glucuronate and L-arabinofuranose residues, and the amount of each depends on the source of xylan: while rice bran xylan is rich in arabinose, birchwood xylan possesses a significant amount of glucuronate and low arabinose content (Kormelink and Voragen, 1993). Acetate groups are not present in softwood xylan (Gregory et al., 1998) (Fig. 1).

The biodegradation of xylan requires thus a set of esterases and glycanases. These enzymes are produced by a number of bacteria and fungi and are mostly

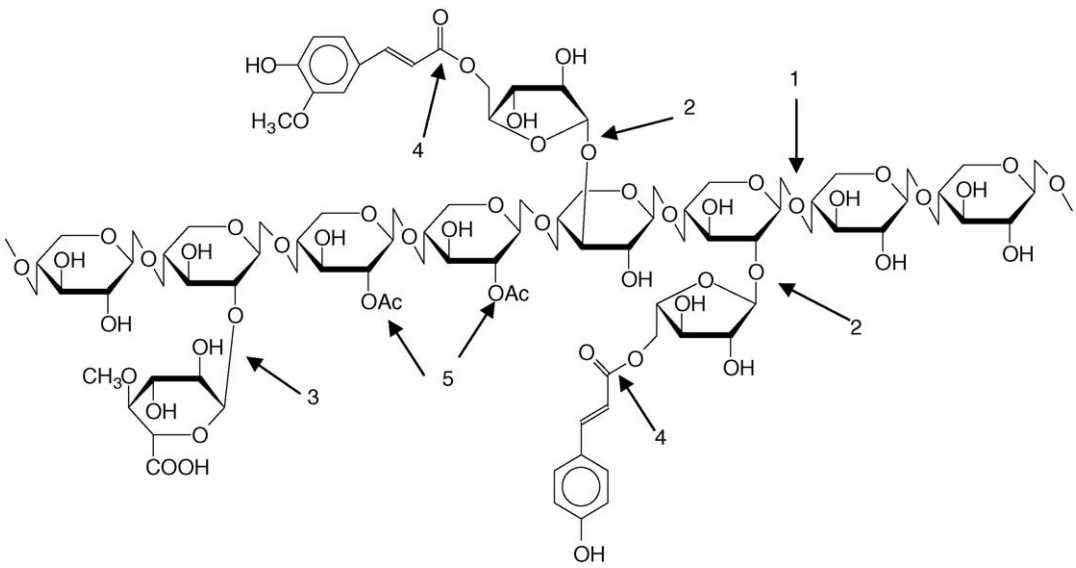

Fig. 1. The structure of xylan and site of action of the enzymes of the xylanase complex. 1: endoxylanases; 2: α-L-arabinofuranosidases; 3: glucuronidases; 4: feruloyl and coumaroyl esterases; 5: acetyl xylan esterases.

extracellular (Sunna and Antranikian, 1997). These enzymes (Fig. 1) are:

- Endoxylanases (E.C. 3.2.1.8); randomly hydrolyze the main chain of xylan, producing a mixture of xylooligosaccharides.
- β-Xylosidases (E.C. 3.2.1.37); liberate xylose from short oligosaccharides.
- α-L-Arabinofuranosidases (E.C. 3.2.1.55); remove L-arabinofuranose side chains.
- α-D-Glucuronidases (E.C. 3.2.1.139); hydrolyze the methyl glucuronate residues.
- Acetyl xylan esterases (E.C. 3.1.1.72); hydrolyze acetate groups from the main chain.
- Feruloyl (E.C. 3.1.1.73) and coumaroyl esterases (E.C. 3.1.1.-); hydrolyze the respective aromatic acids linked to the arabinofuranoside residues.

The known xylanolytic fungi are mostly ascomycetes. Particular attention has been granted to the enzymes produced by *Aspergilli* (de Vries and Visser, 2001) and *Trichoderma* (Wong and Saddler, 1992), while the xylanolytic system of *Penicillia*, also a genus with a large number of species producing xylanases, has not been reviewed.

2. Properties of the xylanases produced by *Penicillia*

The *Penicillia* are mostly saprophytic in nature, and numerous species are of particular value for humans. Probably the best known is *Penicillium notatum*, the producer of the antibiotic penicillin. Also important in the food industry are *P. roqueforti* and *P. camemberti*, associated with the production of particular types of cheese (Moss, 1987).

Many *Penicillia* are soil fungi, and grow in a variety of organic substances, particularly dead plant materials. They produce extracellular hydrolases such as pectic enzymes, lipases, proteases, cellulases and xylanases (Hamlyn et al., 1987).

The production of xylanolytic enzymes by *Penicillia* has been explored in a number of species (see listing in Table 1). Particularly noteworthy is the screening performed by Krogh et al. (2004) who tested 12 strains of different species of *Penicillia* belonging to the subgenera *Biverticillium* (found in plant products related to wood, paper and textiles) and *Furcatum* (present in grassland soils). They observed that the first subgenus strains produced more β-xylosidase, while no differences were detected for endoxylanase. Milagres et al. (1993) have isolated a strain of *P. janthinellum*, which produces high endoxylanolytic activity, while secreting negligible amounts of cellulases. Abdel-Sater and El Said (2001) selected in agar plates containing xylan a large number of fungal isolates from samples containing wheat and rice straw and sugarcane bagasse; among them were five *Penicillium* species, which produced endoxylanases (*P. chrysogenum*, *P. corylophilum*, *P. duclauxii*, *P. funiculosum*, and *P. oxalicum*). Endoxylanase producing *Penicillia* have also been found in a variety of environments: Medeiros et al. (2003) have isolated from the Amazonian forest several *Penicillium* species producing endoxylanase activity. Bradner et al. (1999) have measured hemicellulase activities (mannanase and endoxylanase) from antarctic isolates, among them three *Penicillia*, and found significant endoxylanase activity in *P. hirsutum* and *P. commune* supernatants at 10–20 °C. From all these studies it can be concluded that *Penicillia* constitute a rich source of enzymes for the biodegradation of xylan.

Studies performed on individual xylanolytic enzymes are described below.

2.1. Endoxylanases

This group of enzymes has been widely studied in a variety of fungi and bacteria (Sunna and Antranikian, 1997) including a number of *Penicillium* species. The presence of multiple endoxylanases has been reported in several *Penicillia*. Takenishi and Tsujisaka (1973) isolated from *P. janthinellum* three endoxylanases from extract of wheat bran koji culture. *P. capsulatum* has been shown to produce three endoxylanases; these enzymes have been purified and characterized (Filho et al., 1993; Ryan et al., 2003). Two of these enzymes (XynA and XynB) are produced when the carbon source is a mixture of wheat bran and beet pulp, while XynC is produced in a medium where the carbon source is birchwood xylan. Belancic et al. (1995) purified two endoxylanases from *P. purpurogenum*, and zymograms of supernatants of this fungus (Chávez et al., 2002a) show at least four separate bands. Haas et al. (1992), on the other hand, found no endoxylanase multiplicity in cultures of *P. chrysogenum* grown in oat spelt xylan, and purified, characterized and sequenced the enzyme.

Table 1
Penicillium species, which have been examined for the production of xylanolytic enzymes

Species	Enzymes detected	Best carbon source tried	Reference
P. brasilianum	Endoxylanase	Wet-oxidized wheat straw	Thygesen et al. (2003)
	β-Xylosidase	Wet-oxidized wheat straw	
P. canescens	Endoxylanase	Soya meal + wheat straw	Gaspar et al. (1997)
	Endoxylanase	Wheat straw + xylan	Bakri et al. (2003)
P. capsulatum	Endoxylanase	Beet pulp + wheat bran	Filho et al. (1993)
P. chermisinum	β-Xylosidase	Xylan	Reese et al. (1973)
P. chrysogenum	Endoxylanase	Xylan (oat)	Haas et al. (1992)
	Endoxylanase	Xylan	Abdel-Sater and El Said (2001)
P. commune	Endoxylanase	Cellulose	Bradner et al. (1999)
P. corylophilum	Endoxylanase	Xylan (oat)	Medeiros et al. (2003)
	Endoxylanase	Xylan	Abdel-Sater and El Said (2001)
P. duclauxi	Endoxylanase	Xylan	Abdel-Sater and El Said (2001)
P. expansum	Endoxylanase	Cellulose	Bradner et al. (1999)
P. funiculosum	Endoxylanase	Cellulose + wheat bran	Mishra et al. (1985)
	Endoxylanase	Xylan (oat)	Krogh et al. (2004)
	β-Xylosidase	Xylan (oat)	
	Arabinofuranosidase	Xylan (oat)	
	Endoxylanase	Xylan	Abdel-Sater and El Said (2001)
P. herquei	Endoxylanase	Xylan	Funaguma et al. (1991)
	β-Xylosidase	Xylan (oat)	Ito et al. (2003)
P. hirsutum	Endoxylanase	Cellulose	Bradner et al. (1999)
P. islandicum	β-Xylosidase	Xylan	Reese et al. (1973)
P. janthinellum	Endoxylanase	Xylan (oat)	Milagres et al. (1993)
			Curotto et al. (1994)
	β-Xylosidase	Xylan (oat)	Curotto et al. (1994)
P. kloeckeri	Endoxylanase	Xylan (birchwood)	Haltrich et al. (1996)
P. oxalicum	Endoxylanase	Xylan	Abdel-Sater and El Said (2001)
P. persicinum	Endoxylanase	Xylan (oat)	Krogh et al. (2004)
	β-Xylosidase	Xylan (oat)	
	Arabinofuranosidase	Xylan (oat)	
P. pinophilum	Endoxylanase	Cellulose + barley straw	Brown et al. (1987)
P. purpurogenum	Endoxylanase	Wheat straw	Steiner et al. (1994)
	Acetyl xylan esterase	Acetylated xylan	Egaña et al. (1996)
P. pusillum	β-Xylosidase	Xylan	Reese et al. (1973)
P. roqueforti	Endoxylanase	Xylan (oat)	Medeiros et al. (2003)
P. roseopurpureum	β-Xylosidase	Xylan	Reese et al. (1973)
P. simplicissimum	Endoxylanase	Xylan (oat)	Krogh et al. (2004)
	Arabinofuranosidase	Xylan (oat)	
Penicillium sp. AHT-1	Endoxylanase	Xylan (oat)	Rahman et al. (2003)
	β-Xylosidase		
	Arabinofuranosidase		
Penicillium sp. 40	Endoxylanase	β-Methyl xyloside	Kimura et al. (2000)
P. verruculosum	Endoxylanase	–	Berlin et al. (2000)
P. wortmanni	β-Xylosidase	Xylan	Reese et al. (1973)

Funaguma et al. (1991) have purified an endoxylanase, which has the rather unique property of showing two protein bands of 11,000 and 8300, respectively, on SDS electrophoresis gels, suggesting a protein made of different subunits; the molecular weight of the native enzyme, however, has not been determined. Kimura et al. (2000) have isolated an acidophilic endoxylanase from *Penicillium* sp. 40, which can grow in acidic conditions, such as pH 2.0.

The main properties of purified endoxylanases from *Penicillia* are listed in Table 2.

2.2. β-Xylosidases

Reese et al. (1973) tested 250 microorganisms (bacteria and fungi) for their ability to secrete β-xylosidases. Of the 11 most active producers (all fungi), five were *Penicillia*, and the most active fungus found was *P. wortmanni*. Best yields were obtained when the fungus was grown on xylan in the presence of Tween 80 and a mixture of methyl-β-D- and methyl-α-D-xylopyranosides. *P. funiculosum* (Mishra et al., 1985) and *P. janthinellum* (Curotto et al., 1994) have also been described to be good producers of β-xylosidase. Ito et al. (2003) have identified and purified two β-xylosidases liberated from the cell surface of *P. herquei*. The enzyme from *P. wortmanni* was purified and characterized by Deleyn et al. (1978). Table 3 shows some properties of β-xylosidases isolated from *Penicillia*.

2.3. Arabinofuranosidases (AF)

Beldman et al. (1997) has proposed a classification of the arabinanases (a generic term to describe all enzymes which act on polysaccharides containing arabinose residues) based on their mode of action, distinguishing six classes. Of those, three are arabinofuranosidases. They are all exo-type enzymes, which hydrolyze non-reducing arabinofuranose residues. α-L-Arabinofuranosidases type A act on short oligosaccharides. Type B α-L-arabinofuranosidases are capable of hydrolyzing side-chain arabinose residues from polymeric substrates. Both groups are also recognized for their ability to hydrolyze the synthetic substrate *p*-nitrophenyl α-L-arabinofuranose (pNPA). The third type, class C, does not act on pNPA, but is specific for arabinoxylan. Enzymes from the three classes have been found in culture supernatant of various fungi.

Table 2
Properties of endoxylanases from *Penicillium* species

Source	Enzyme	MW ($\times 10^{-3}$)	p*I*	pH optimum	Temperature optimum (°C)	Reference
P. brasilianum		31	>9	–	–	Jørgensen et al. (2003)
P. canescens	Xyl-31	31	8.2–9.3	5.9	55	Sinitsyna et al. (2003b)
P. capsulatum	XynA	28.5	5.0–5.2	4.0	47–48	Filho et al. (1993)
	XynB	29.5	5.0–5.2	4.0	47–48	
	XynC	22	2.8	3.8	48	Ryan et al. (2003)
P. chrysogenum		35	4.2	6.0	40	Haas et al. (1992)
P. funiculosum	XynC	23.6	3.7	5.0	55	Furniss et al. (2002)
P. herquei		See text	5.1	3.0	50	Funaguma et al. (1991)
P. janthinellum	I	–	–	5.3	–	Takenishi and Tsujisaka (1973)
	II	–	–	4.7	–	
	III	–	–	4.7	–	
P. purpurogenum	XynA	33	8.6	7.0	60	Belancic et al. (1995)
	XynB	23	5.9	3.5	50	
Penicillium sp. 40	XynA	25	4.7	2.0	50	Kimura et al. (2000)
Penicillium sp. AHT-1		21	–	6.0	50	Rahman et al. (2003)
P. verruculosum	XYN	65	4.5	–	–	Berlin et al. (2000)

Table 3
Properties of β-xylosidases from *Penicillium* species

Source	Enzyme	MW ($\times 10^{-3}$)	p*I*	pH optimum	Temperature optimum (°C)	Reference
P. herquei	SI	103.7	–	4.0	50	Ito et al. (2003)
	S2	37.46	–	6.5	30	
P. wortmanni		100	5.0	3.3–4	–	Deleyn et al. (1978)

Table 4 summarizes the properties of arabinofuranosidases isolated and studied from *Penicillia*. Filho et al. (1996) have purified two arabinofuranosidases from *P. capsulatum* solid-state cultures. These enzymes are type B AFs based on their substrate specificity. *P. purpurogenum* also produces several isoenzymes of AF (De Ioannes et al., 2000); three different forms have been separated by isoelectrofocusing and detected by zymograms. One of these enzymes has been purified and sequenced (Carvallo et al., 2003). It can also be classified as a B-type enzyme (it acts on pNPA and releases arabinose from oat spelts xylan) and its sequence shows that the enzyme belongs to family 54 of the glycosyl hydrolases.

Five distinct arabinan-degrading enzymes have been found to be secreted by *P. chrysogenum*. Among them are two AFs, identified as AFS1 and AFQ1, which have been purified and characterized (Sakamoto and Kawasaki, 2003). Both enzymes are type B as established by their substrate specificity; based on a short amino terminal sequence, they can be classified respectively as family 51 (AFQ1) and family 54 (AFS1) of the glycosyl hydrolases. Two other arabinanases secreted by this fungus are an exo-arabinanase (Sakamoto and Thibault, 2001), which releases arabinobiose from α-L-arabinan and an endo-arabinanase (family 43), which does not act on arabinoxylan or pNPA (Sakamoto et al., 2003).

Sinitsyna et al. (2003a) have isolated the major components of the xylanase system of *P. canescens*. Among these enzymes are two arabinofuranosidases (called AF-60 and AF-70). The enzymes are active against pNPA, arabinoxylan and arabinan, suggesting that they are type B enzymes. They show, however, some differences in substrate specificity: AF-60 is active on glucuronoxylan while AF-70 acts on xyloglucan.

2.4. α-Glucuronidases

They are probably the less studied of the xylan debranching enzymes. Although they have been isolated from several fungi and bacteria (see listing in Biely et al., 2000), they have not as yet been described in *Penicillium*.

2.5. Acetyl xylan esterases (AXEs)

Little work has been done on AXEs from *Penicillium*. Egaña et al. (1996) have purified and characterized two AXEs from *P. purpurogenum* (see Table 5). More recently a third AXE has been isolated from the same organism (Fuentes, unpublished). All three enzymes show very similar substrate specificity, so it is not clear at present why this organism needs to produce such a variety of AXEs, an unprecedented finding in xylanolytic fungi.

Table 4
Properties of arabinofuranosidases from different *Penicillium* species

Source	Enzyme	MW ($\times 10^{-3}$)	p*I*	pH optimum	Temperature optimum (°C)	Reference
P. capsulatum	AraI	64.5	4.15	4.0	60	Filho et al. (1996)
	AraII	62.7	4.54	4.0	55	
P. canescens	AF-60	60.0	7.6	4.7	70	Sinitsyna et al. (2003a)
	AF-70	70.0	3.8–4.0	5.5	70	
P. chrysogenum	AFQ1	79.0	–	4.0–6.5	–	Sakamoto and Kawasaki (2003)
	AFS1	52.0	–	3.3–5.0	–	
P. purpurogenum	ABF 1	58.0	6.5	4.0	50	De Ioannes et al. (2000)

Table 5
Properties of acetyl xylan esterases from *Penicillium* species

Source	Enzyme	MW ($\times 10^{-3}$)	p*I*	pH optimum	Temperature optimum (°C)	Reference
P. purpurogenum	AXE I	48	7.5	5.3	50	Egaña et al. (1996)
	AXE II	23	7.8	6.0	60	
	AXE III	85.7	6.3	5.3	20	Fuentes (unpublished)

2.6. Feruloyl and coumaroyl esterases

Based on substrate utilization, induction medium and supported by amino acid sequence identities, these enzymes have been classified in four classes (Crepin et al., 2004). Model substrates have been used for the classification: methyl ferulate (MFA), methyl caffeate (MCA), methyl sinapate (MSA) and methyl coumarate (MpCA). Type A enzymes are induced preferentially by wheat bran and other cereal-derived substrates and act mainly on MFA, MSA and MpCA and show amino acid sequences similarity to lipases; type B enzymes are mainly induced by sugar beet pulp; they are similar to family 1 acetyl xylan esterases and utilize in preference MFA, MpCA and MCA. Enzymes of type C are induced by both wheat bran and sugar beet pulp, act on all four substrates and show sequence similarity to tannases and chlorogenate esterases. Those of family D act also on all four methyl esters, are induced mainly by wheat bran and show sequence similarity to endoxylanases. Enzymes A and D also release diferulic acid from cereal cell walls.

Feruloyl esterases have been studied in several *Penicillium* species. The properties of these enzymes have been summarized in Table 6. Castanares et al. (1992) purified a feruloyl/coumaroyl esterase from *P. pinophilum*. The fungus was grown in solid state in an oat straw/wheat bran mixture; it hydrolyzes MFA and MpCA, and it is also active on acetyl xylan and pNPA, but no sufficient information (such as amino acid sequence) is available to allow a family classification. Feruloyl and *p*-coumaroyl esterase activities have been detected in cultures of *P. expansum* and *P. brevicompactum* (Donaghy and McKay, 1995) using methyl ferulate as main carbon source in liquid cultures and sugar beet pulp and wheat bran in solid state cultures, the latter being a better inducer of activity. Highest activity was found in the *P. expansum* culture, and this enzyme was purified and characterized (Donaghy and McKay, 1997). Based on its substrate specificity (it acts on MFA, MpCA and MCA, but not on MSA) and preferred induction medium, it can be classified as a type B enzyme. More thoroughly studied is the enzyme from *P. funiculosum* (Kroon et al., 2000) obtained from sugar beet pulp cultures. It is a typical type B enzyme. Its sequence is known and it possesses a cellulose-binding module.

3. Genes encoding xylanolytic enzymes from *Penicillium*

3.1. Endoxylanases

The most popular classification of the glycosyl hydrolases, based on sequence similarities, is that presented in the CAZY database (http://afmb.cnrs-mrs.fr/CAZY/; Henrissat and Davies, 1997). Enzymes with endoxylanase activity have been found in families 5, 7, 8, 10, 11 and 43 (Collins et al., 2005), most commonly in families 10 and 11. A search of the database and reference sources shows the existence of

Table 6
Properties of feruloyl/coumaroyl esterases from *Penicillium* species

Source	Enzyme	MW ($\times 10^{-3}$)	p*I*	pH optimum	Temperature optimum (°C)	Reference
P. pinophilum		57	4.6	6.0	55	Castanares et al. (1992)
P. expansum		57.5	–	5.6	37	Donaghy and McKay (1997)
P. funiculosum	FAEB	53	6.0	–	–	Kroon et al. (2000)

Table 7
Sequenced xylanolytic genes from *Penicillium*

Enzymatic activity	Source	Gene	Glycosyl hydrolase family	Reference
Endoxylanase	*P. funiculosum*	*xynA*	7	Alcocer et al. (2003)
		xynB	11	Alcocer et al. (2003)
		xynC	11	Furniss et al. (2002)
		xynD	10	Furniss et al. (2005)
	P. purpurogenum	*xynA*	10	Chávez et al. (2001a)
		xynB	11	Díaz et al. (1997)
	P. chrysogenum	*xylP*	10	Hass et al. (1993)
	P. canescens	*xylA*	10	Serebryanyi et al. (2002)
	P. simplicissimum	*xynA*	10	Schmidt et al. (1998)
	Penicillium sp. 40	*xynA*	11	Kimura et al. (2000)
β-xylosidase	*P. herquei*	*s2*	43	Ito et al. (2003)
	P. purpurogenum[a]	–	43	Ito et al. (2003)
Arabinofuranosidase	*P. purpurogenum*	*abfl*	54	Carvallo et al. (2003)
Enzymatic activity	**Source**	**Gene**	**Carbohydrate esterase family**	**Reference**
Acetyl xylan esterase	*P. purpurogenum*	*axell*	5	Gutiérrez et al. (1998)
		axel	1	Gordillo (unpublished)
Feruloyl esterase	*P. funiculosum*	*faeA*	1	GenBank AJ312296
		faeB	1	Kroon et al. (2000)

[a] Only a partial sequence of 290 bp is available.

10 sequences for *Penicillium* endoxylanases, five from family 10, four from family 11 and one belonging to family 7 (Table 7).

P. funiculosum is the organism from which the most number of sequenced endoxylanases genes (four) have been described, three of them encoding the only known modular endoxylanases from *Penicillium* with carbohydrate-binding modules (CBM): The *xynA* gene (coding for a xylanase/cellobiohydrolase enzyme with similarity to family 7 cellobiohydrolases); the *xynB* gene (belonging to family 11) and the *xynD* gene (belonging to family 10). All these enzymes include a catalytic domain, a Ser/Thr-rich linker and a putative CBM (Alcocer et al., 2003; Furniss et al., 2005). The *xynC* gene encodes a non-modular endoxylanase with significant sequence similarity to family 11 glycosyl hydrolases (Furniss et al., 2002).

In *P. purpurogenum*, two endoxylanases genes have been reported: the *xynA* gene, belonging to family 10 (Chávez et al., 2001a), and *xynB*, belonging to family 11 (Díaz et al., 1997; see also GenBank AJ359553). None of these endoxylanases have CBMs, but it is important to remark that from the zymograms showed by Chávez et al. (2002a), it is possible to deduce that *P. purpurogenum* produces several other endoxylanases of higher molecular weight, which may contain CBMs in their structure.

One endoxylanase gene from each, *P. chrysogenum*, *P. simplissicimum* and *P. canescens*, has been sequenced, all belonging to family 10: the *xylP* gene (Hass et al., 1993), the *xynA* gene (Schmidt et al., 1998), and the *xylA* gene (Serebryanyi et al., 2002), respectively. In addition, one acidophilic endoxylanase gene belonging to family 11 of the endoxylanases, *xynA*, has been found in *Penicillium* sp. 40 (Kimura et al., 2000).

Except for *xynA* from *P. funiculosum*, all the genes described above contain introns. As a rule, family 11 endoxylanase genes (*xynA* and *xynB* from *P. funiculosum*, *xynB* from *P. purpurogenum* and the enzyme from *Penicillium* sp. 40) contain one intron (Díaz et al., 1997; Kimura et al., 2000; Furniss et al., 2002; Alcocer et al., 2003). On the other hand, family 10 endoxylanase genes (*xynA* from *P. purpurogenum* and the enzymes from *P. chrysogenum*, *P. canescens* and *P. simplissicimum*), contain eight to nine introns (Hass et al., 1993; Schmidt et al., 1998; Chávez et al., 2001a; Serebryanyi et al., 2002). A special case is *xynD* from *P. funiculosum*, which belongs to family 10 but contains just three introns (Furniss et al., 2005). The significance of these findings remains unknown.

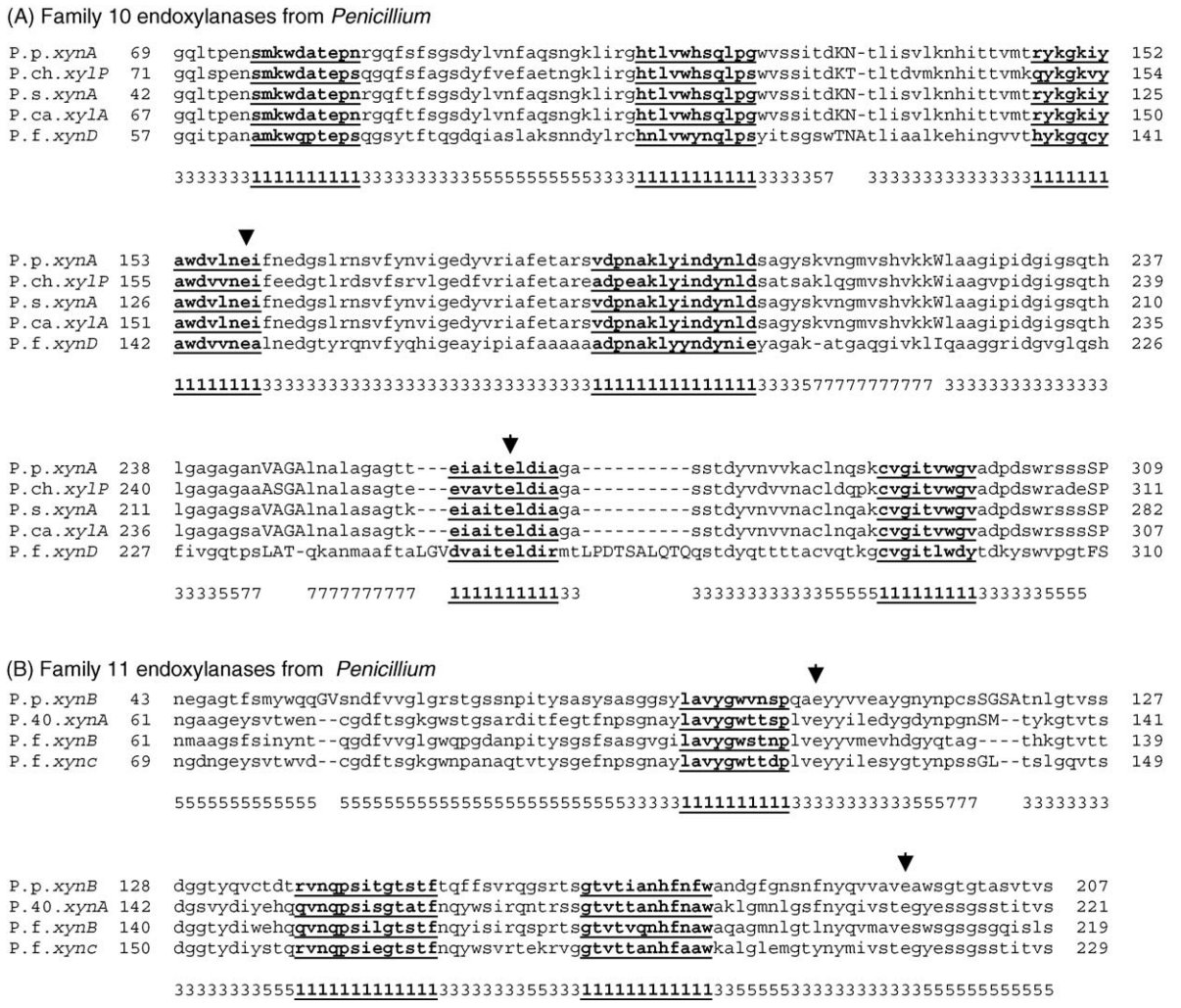

Fig. 2. Sequence alignment of *Penicillium* family 10 (A) and family 11 (B) endoxylanases using MATCHBOX. Numbers under the boxes indicate degree of similarity (1 is highest; the boxes are in bold and underlined). Only segments of the protein sequences with high similarity are included. Numbers at the left and right of each sequence indicate the protein segment aligned. The postulated catalytic glutamic acids are shown by arrows. P.p.: *P. purpurogenum*; P.ch.: *P. chrysogenum*; P.s.: *P. simplicissimum*; P.ca.: *P. canescens*; P.f.: *P. funiculosum*; P.40: *Penicillium* sp. 40. References for these sequences are given in Table 7.

Some structural relationships between endoxylanases from *Penicillium* were established by protein sequence alignments using the MATCHBOX program (Depiereux and Feytmans, 1992). This program matches the most similar nine-residue segments in a scan of the whole set of sequences. Thus, MATCHBOX seeks for "similarity boxes", emphasizing the sequence similarities among the aligned enzymes. Using this computer program, sequences belonging to family 10 and family 11 were aligned separately (Fig. 2). In the alignment performed for family 10 (Fig. 2A), five boxes of very high similarity are detected (value of 1 in MATCHBOX), which include the two conserved catalytic glutamate residues (Moreau et al., 1994; Schmidt et al., 1998). The rest of the sequence alignment shows regions of medium or low similarity. An interesting

fact is observed when the regions of highest and lowest similarity are superimposed on the available tertiary structure of endoxylanase from *P. simplissicimum*, an (α/β) barrel (Schmidt et al., 1998). The regions of highest similarity (the most conserved regions among the sequences) are located in the center of the barrel and contain the glutamate residues from the active site, while the most variable regions (MATCHBOX lowest similarity score) are found in the periphery of the structure, preferentially in the α-helices (Chávez et al., 2001a). This observation suggests that the structure of family 10 endoxylanases from *Penicillium* (and probably from other fungi) have a highly conserved central core sheltering the active site, the rest of the structure being mostly variable.

The alignment of family 11 (shown in Fig. 2B) is less informative. Although three boxes of very high similarity are detected by MATCHBOX, the conserved catalytic glutamate residues are found outside of these conserved regions. The rest of the sequence shows regions of medium or low similarity.

3.2. β-Xylosidases

β-Xylosidases are found in families 3, 39, 43, 51, 52 and 54 of the glycosyl hydrolases, but fungal enzymes have been described so far only for families 3, 43 and 54.

Only one β-xylosidase complete gene sequence from *Penicillium* is found in the databases (Table 7). This gene from *P. herquei* IFO 4674, named *s2*, consists of a 1005 base pair (bp) sequence. It contains no introns and encodes a 335 amino acid protein with no apparent signal peptide, in spite of the fact that this enzyme is a cell surface associated protein (Ito et al., 2003). Besides this sequence, a 290 bp partial sequence of a β-xylosidase from *P. purpurogenum* has been reported (Ito et al., 2003). Both enzymes present a high degree of similarity with family 43 glycosyl hydrolases. It is interesting to remark that most fungal β-xylosidases from organisms with phylogenetic proximity to *Penicillium* (such as *Aspergillus*) are found in family 3 of the glycosyl hydrolases, but not in family 43, which contains mainly xylosidases from bacterial origin. However, some fungi whose entire genome was recently sequenced have open reading frames (ORFs) that encode for hypothetical proteins with high similarity to the *Penicillium* β-xylosidases described above: *A. nidulans* (accession number XP_405614 in NCBI Protein Database), *Magnaphorte grisea* (accession number XP_366835 in NCBI Protein Database) and *Neurospora crassa* (Galagan et al., 2003). In addition, a partial sequence of a β-xylosidase from *Aspergillus oryzae* (Ito et al., 2003) and the β-xylosidase from *Cochliobolus carbonum* (Wegener et al., 1999) belong also to family 43. These data suggest that there are two structurally different fungal β-xylosidases, which are grouped into two different glycosyl hydrolase families (3 and 43).

3.3. α-Arabinofuranosidases

Arabinofuranosidases are found in families 43, 51, 54 and 62 of the glycosyl hydrolases.

The recently sequenced *abf1* gene from *P. purpurogenum* is the only α-arabinofuranosidase sequence available from a *Penicillium* (Carvallo et al., 2003; see Table 7). This gene has an open reading frame of 1518 bp, it does not contain introns and encodes for a protein of 506 amino acids, with a calculated molecular mass of 49.6 kDa. This gene belongs to family 54 and has very high similarity (74–81%) with α-arabinofuranosidases from *Aspergillus* and *Trichoderma* species (Carvallo et al., 2003).

In addition to the α-arabinofuranosidase described above, it is interesting to note that a gene coding for an exo-1,5-α-arabinanase (a related arabinan-degrading enzyme which releases arabinobiose from α-L-arabinan; Sakamoto and Thibault, 2001) has been cloned from *P. chrysogenum*, and sequenced (Sakamoto et al., 2004). This enzyme does not show sequence similarity to other arabinan degrading enzymes.

3.4. Acetyl xylan esterases

Acetyl xylan esterases have been found in families 1–7 of the carbohydrate esterases.

From the genus *Penicillium*, only two genes from *P. purpurogenum* have been cloned and sequenced (Table 7). Unpublished data from Gordillo and Eyzaguirre indicate that the *axeI* gene (see GenBank AF529173) presents an open reading frame of 1278 bp, including two introns of 68 and 61 bp, and codes for a mature protein of 351 amino acids. This gene has a modular structure: a catalytic module at the amino

terminus belonging to family 1 of the carbohydrate esterases, a linker rich in serines and threonines, and a family 1 carboxy terminal CBM, which is similar to that of acetyl xylan esterase from *T. reesei* and binds the enzyme to cellulose but not xylan.

The *axeII* gene from *P. purpurogenum* was the first *axe* cloned and sequenced from a *Penicillium* (Gutiérrez et al., 1998). This gene has an open reading frame of 831 bp and contains two introns, giving a mature protein of 207 residues. Interestingly, this gene lacks the linker and carbohydrate-binding module described in the *axeI* gene; these are present, however, in the *axeI* gene from *Trichoderma reesei*. Although AXE II from *P. purpurogenum* shows a 66% identity with the catalytic domain of AXE from *T. reesei* (Gutiérrez et al., 1998), the lack of CBM in the former indicates a different evolutionary path for both enzymes, the *T. reesei* AXE having acquired its CBM by gene fusion. The existence of several AXEs suggests a possible complementary role in ester hydrolysis during xylan degradation in *P. purpurogenum.*

3.5. Feruloyl esterases

Feruloyl esterases have been described only in family 1 of the carbohydrate esterases.

Two genes encoding feruloyl esterases from *P. funiculosum*, *faeA* (see GenBank AJ312296) and *faeB* (Kroon et al., 2000) have been sequenced (Table 7). The *faeB* gene is contained in an 1120-bp ORF, which is interrupted by one intron and encodes a putative 335-residue mature protein. The protein FaeB is composed of two distinct domains separated by a 20-amino acid linker region rich in Thr, Ser and Pro: a catalytic N-terminal domain 276 amino acid long, and a 39 amino acid C-terminal domain which closely resembles the family 1 CBMs of fungal glycosyl hydrolases (Kroon et al., 2000). The analysis of the other feruloyl esterase gene from *P. funiculosum*, *faeA*, reveals that this gene has an ORF of 1096 bp, which is interrupted by one intron and encodes a mature protein of 325-residues. When the deduced protein sequence from the *faeA* gene is submitted to BLAST, no putative conserved domain resembling a known CBM is found. This finding points to a possible complementary role of FaeA and FaeB in feruloyl residue hydrolysis during xylan degradation by *P. funiculosum*.

4. Genetic organization of *Penicillium* xylanolytic genes

In filamentous fungi, several metabolic pathways are organized as gene clusters (Keller and Hohn, 1997). Usually, genes that show this kind of organization encode secondary metabolism genes, such as penicillin producing genes (Diez et al., 1990; Laich et al., 2002). In other cases, the genes of some metabolic pathways that are activated under specific growth conditions, such as nutrient deprivation or competition with other microorganisms, have been shown to be organized as clusters. The ethanol utilization or nitrate assimilation pathways are good examples (Johnstone et al., 1990; Fillinger and Felenbok, 1996). On the other hand, most of the complex fungal degradation systems have as a common characteristic the presence of multiple enzyme forms, whose physiological function are not well understood. Several of these systems appear to be organized as gene clusters. For example, it has been found that some ligninolytic fungi have multiple ligninase genes organized as clusters (Gaskell et al., 1991; Johansson and Nyman, 1996; Stewart and Cullen, 1999). In cellulolytic systems, a cluster of cellobiohydrolase genes has been described in *Phanerochaete chrysosporium* (Covert et al., 1992), and in *Trichoderma reesei*, three cellulolytic genes (*cbh1*, *ccb2* and *egl2*) are located on the same chromosome (Mantyla et al., 1992).

The first approach to analyze the genetic organization of xylanolytic genes in fungi was performed by Carter et al. (1992) in *T. reesei*. By pulse-field gel electrophoresis, these authors resolved the chromosomes of *T. reesei* strain QM6a and several of its derivatives. Using Southern blots with different probes, several genes were mapped. The endoxylanase genes *xyl1* and *xyl2* were found to be located in the same chromosome, but the evidence showed that these genes were not physically close. In the case of *Penicillia*, this question has been studied so far only in *P. purpurogenum*: Chávez et al. (2002b) showed that the transformation of a *Saccharomyces cerevisiae* strain with genomic fungal DNA from *P. purpurogenum* yields a yeast strain that is able to produce endoxylanase and β-xylosidase activities. This suggested that the xylanolytic system of *P. purpurogenum* could be organized as a gene cluster, in a similar way to the ligninolytic or cellulolytic clusters described above. To test this hypothesis, the

chromosomes from *P. purpurogenum* were separated by pulse-field gel electrophoresis and hybridized to five available probes for xylanolytic genes from this fungus: two endoxylanases, two acetyl xylan esterases and one α-arabinofuranosidase (Chávez et al., 2001b). With this approach and other complementary experiments, it was concluded that the xylanolytic system of *P. purpurogenum* is widely distributed in the genome of this fungus.

The results of Carter et al. (1992) on *T. reesei* and of Chávez et al. (2001b) on *P. purpurogenum* have important implications in the evolutionary acquisition of the xylanolytic system in *Penicillium* and other fungal species, indicating that this system was not incorporated as a "xylanolytic cassette" in a single event. On the contrary, probably the xylanolytic system of these organisms was developed by the acquisition of genes in different events. Given the scarce amount of work reporting the chromosomal location of fungal xylanases, it is not possible to extend these observations to other fungal xylanolytic systems, and further work is necessary to establish a possible general rule.

5. Regulation of the expression of xylanases in *Penicillium*

Molecular mechanisms that control the regulation of xylanase gene expression are still poorly understood. Studies on the subject have been carried out in several fungi, mainly *Aspergillus* and *Trichoderma* (Mach et al., 1996; Zeilinger et al., 1996; Orejas et al., 1999; Xu et al., 2000).

The regulation of xylanase genes in *Penicillia* has been studied chiefly indirectly by studying the use of different carbon sources and growth conditions on enzyme production. For example, Jørgensen et al. (2004) recently showed that three *Penicillia* (*P. pinophilum*, *P. persicinum* and *P. brasilianum*) were stimulated for β-xylosidase and endoxylanase production by the presence of xylose as carbon source, while glucose acted as repressor. Using the same organisms, Jørgensen et al. (2005) showed that xylan (from both birchwood and oat spelts) induced endoxylanase production but the three species showed differences in the magnitude of the response. In *P. purpurogenum* an enhanced production of endoxylanase, acetyl xylan esterase and arabinofuranosidase activities has been observed when the fungus is grown on specific substrates such as wheat straw, acetylated oat spelt xylan and arabitol, respectively, in preference to other substrates such as birchwood xylan or oat spelt xylan (Eyzaguirre et al., 1992; Egaña et al., 1996; De Ioannes et al., 2000). In *P. funiculosum*, xylanases are produced in a medium containing either xylan or carboxymethyl cellulose (Rao et al., 1988), but the highest production was obtained when raw substrates (sugar cane bagasse, rice straw and wheat straw) were used (Fadel and Fouda, 1993). In *P. canescens*, differences in endoxylanase production have been observed under different carbon and nitrogen sources; among several agricultural wastes tested, untreated wheat straw gave highest titers (Bakri et al., 2003). In the case of *P. janthinellum*, Curotto et al. (1994) showed differences in xylanase production both by carbon source and pH. *Penicillium* sp. 40 is also clearly influenced by pH, because its xylanase A is preferentially produced at acidic pH (Kimura et al., 2000). All these examples suggest that the induction of the expression of the xylanolytic enzymes in *Penicillium* is regulated at the genetic level by a complex set of substrate and environmental conditions.

5.1. Glucose repression

A common finding in filamentous fungi including *Aspergilli* and *Trichoderma* (Prade, 1995) is that the production of the xylanolytic enzymes is repressed by glucose (carbon catabolite repression). In *Penicillium* species, glucose repression has been demonstrated by northern analysis for several xylanases from *P. purpurogenum* (Chávez et al., 2002a; Carvallo et al., 2003; Chávez et al., 2004), and in the *faeB* gene from *P. funiculosum* (Kroon et al., 2000). In *P. chrysogenum*, Zadra et al. (2000) showed by northern analysis that the expression of the xylanase gene *xylP* is transcriptionally repressed by glucose; this was confirmed by the use of a β-glucuronidase (*uidA*) reporter gene fused with the *xylP* promoter.

At the molecular level, glucose repression of xylanases has been associated with the presence of binding sites for CreA in their promoters. CreA is a negatively acting protein that mediates carbon catabolite repression in *Aspergilli* and *Trichoderma* (Cubero and Scazzocchio, 1994). In xylanase promoters from *Penicillium*, sites for the binding of CreA have been

described in the promoter of *xylP* from *P. chrysogenum* (Zadra et al., 2000), *xylA* from *P. canescens* (Serebryanyi et al., 2002), *faeB* from *P. funiculosum* (Kroon et al., 2000) and in all the sequenced promoters studied thus far from xylanases of *P. purpurogenum*: *xynA*, *xynB*, *axeII* and *abf1* (Chávez et al., 2002a; Carvallo et al., 2003; Chávez et al., 2004). In addition to these published data, a search for CreA binding sites in other *Penicillium* xylanase promoters released in GenBank, shows the existence of CreA binding sites in the *axeI* promoter from *P. purpurogenum* (GenBank AF529173), in the *s2* promoter from *P. herquei* (GenBank AB093564), in the promoter of the acidic *xynA* gene from *Penicillium* sp. 40 (GenBank AB035540) and in the promoters of *xynA*, *xynB*, *xynC* and *faeA* genes from *P. funiculosum* (GenBank AJ312295, AJ489605, AJ278385 and AJ312296, respectively). Also, a putative sequence for CreA binding was found at −7 bp from the start codon in *xynD* gene in *P. funiculosum* (GenBank AJ634957), but due to its location (downstream from the putative TATA box and next to the start codon), its functionality is uncertain. Although CreA-like proteins have not been specifically described so far in *Penicillia*, the results presented above suggest that a similar repression mechanism is operative in this fungal genus as it is in *Trichoderma* and *Aspergilli*.

5.2. Induction of the expression of xylanolytic genes

The transcriptional activator XlnR is the main regulatory element that has been proposed to participate in xylanase gene expression (van Peij et al., 1998). Initially found in *Aspergillus*, the presence of sites for its binding has been described in almost all the sequenced xylanase promoters from the *Penicillium* species studied thus far.

Usually, only one XlnR binding-site is present for each xylanolytic gene in *Penicillium*. This situation has been found in the *xylA* gene from *P. canescens* (Serebryanyi et al., 2002), in *xynA*, *xynB* and *faeB* genes from *P. funiculosum* (Alcocer et al., 2003; Kroon et al., 2000) and in *xynA*, *axeI*, *axeII*, and *abf1* genes from *P. purpurogenum* (Chávez et al., 2002a; Carvallo et al., 2003; Chávez et al., 2004; see GenBank AF529173 for the *axeI* promoter). However, there are a few cases of xylanolytic genes with more than one XlnR binding site in their promoter. These are the *xylP* promoter from *P. chrysogenum* (Zadra et al., 2000) and the *xynC* promoter from *P. funiculosum* (GenBank AJ278385), where the analysis of their sequence revealed the presence of three XlnR binding sites. This multiplicity is also observed in endoxylanase gene promoters from *Aspergilli*: the *xlnD* promoters from *A. niger* (GenBank Z84377), *xlnB* from *A. tubingensis* (van Peij et al., 1998) and *xynB* from *A. kawachii* (GenBank D38070) contain two XlnR binding sites while the *xynC* promoter from *A. kawachii* (GenBank D14848) has three. An unusual case is the promoter of *xynB* from *P. purpurogenum*, which shows the presence of eight XlnR binding sites These eight sites are repeated in tandem and separated by a conserved interbox region of 12 bp, all in a DNA segment comprising just 138 bp (Chávez et al., 2002a). The presence of more than one XlnR binding site in xylanolytic genes has been related to enhanced expression levels. In fact, it has been demonstrated that both *xylP* from *P. chrysogenum* and *xynB* from *P. purpurogenum* are highly expressed genes (Zadra et al., 2000; Chávez et al., 2002a). Thus, the presence of repetitive XlnR sequences in these promoters may be an evolutionary strategy to enhance expression (Zadra et al., 2000; Chávez et al., 2002a).

Despite the fact that all the promoters previously described have similar binding sequences for XlnR, some differences in the expression of their genes are observed. For example, in *P. purpurogenum*, oat spelts xylan (an arabinoxylan containing 9.7% arabinose and 4.3% uronic acids; Kormelink and Voragen, 1993) induces the expression of *xynA*, *xynB* and *axeII* genes; on the contrary, birchwood xylan (a glucuronoxylan, which contains 1% arabinose and 8.3% uronic acids; Kormelink and Voragen, 1993) induces the expression of *xynB* and *axeII* genes, but not the expression of *xynA* (Chávez et al., 2002a; Chávez et al., 2004). When xylose and xylitol (simple sugars products of xylan degradation) were used as substrates for induction, a low but detectable level of expression of the *xynB* and *axeII* enzymes was observed, but expression *of xynA* was again not detected (Chávez et al., 2002a; Chávez et al., 2004). In the fungus *P. chrysogenum*, *xylP* gene expression is induced by oat spelt xylan and xylose (Hass et al., 1993; Zadra et al., 2000), indicating a similar behavior to that observed for *xynB* or *axeII* in *P. purpurogenum*. These data, obtained from the analysis of expression of *Penicillium* xylanase genes, suggest

the existence of two kinds of enzymes: a first group, which is induced both by polymeric and monomeric substrates, and a second group whose expression is restricted to induction by complex substrates, thus suggesting a different role for these xylanases in xylan degradation.

5.3. Regulation of xylanase expression by pH

The regulatory factor PacC (Tilburn et al., 1995) has been involved in the pH regulation of several fungal genes (Denison, 2000), and binding sites for this protein have been described in promoters of *xynA*, *abf1*, *axeI* and *axeII* from *P. purpurogenum* (Chávez et al., 2002a; Carvallo et al., 2003; Chávez et al., 2004) and in the *xylP* promoter from *P. chrysogenum* (Zadra et al., 2000). Also, potential binding sites for this regulatory element are present in some xylanase promoters registered in the GenBank database: *axeI* from *P. purpurogenum* (AF529173), *xynC* and *faeB* from *P. funiculosum* (AJ278385 and AJ291496) and the acidic *xynA* from *Penicillium* sp. 40 (AB035540).

The influence of pH in xylanase expression is mixed. Steiner et al. (1994) showed that high xylanolytic activities are detected at neutral pH in *P. purpurogenum*, but not at acidic or alkaline pHs, where only a residual activity was recorded. According with this observation, the growth of *P. purpurogenum* at these marginal pHs in media containing xylan is reduced by about 10–40% (Chávez et al., 2002a). This was confirmed at the molecular level by northern blot experiments carried out at different pH values. The transcripts of *xynA*, *xynB*, *abf1* and *axeII* genes were observed only at neutral pH (about 6.6) but not at acidic or alkaline pHs (Chávez et al., 2002a; Carvallo et al., 2003; Chávez et al., 2004). This substantiates that all these enzymes are tightly regulated by pH, expressing their transcripts at neutral pH only. Similar observations were made by Brown et al. (1987) using enzymatic measurements in *P. pinophilum*, which also produces its xylanases at neutral pH, but in this case no confirming molecular studies were performed.

In *P. janthinellum*, on the other hand, a β-xylosidase activity was found only at pH 4.5. At the same pH, endoxylanase production was also enhanced, indicating that in this fungus the xylanase expression occurs at slightly acidic pH (Curotto et al., 1994). More dramatic is the case of the endoxylanase A (XynA) from *Penicillium* sp. 40, a fungus, which can grow in highly acidic conditions. The production of XynA in this fungus was increased in acid medium, and the enzyme is stable at pH 2.0–5.0 with an optimum pH at 2.0 (Kimura et al., 2000), suggesting regulation by pH of this gene.

The above cases are examples of a dependence of xylanase expression from pH. However, in the case *P. chrysogenum*, its *xylP* gene seems to be insensitive to pH, because its expression is independent of the environmental pH, despite the fact that its promoter contains two sites for PacC binding (Zadra et al., 2000). Other interesting and singular case is the effect of pH on the expression of the *xynB* gene from *P. purpurogenum*. The promoter of *xynB* lacks putative PacC binding sites; however, this gene is tightly regulated by pH (Chávez et al., 2002a). The cases of *xylP* and *xynB* indicate that the role of PacC in pH regulation of *Penicillium* xylanases is not clear; pH regulation does not occur in *xylP* despite the presence of putative PacC binding sites, and, on the other hand, *xynB* is regulated by pH despite the lack of these sites. Disagreements in pH regulation have also been reported in other *Penicillium* PacC-containing genes, such as the penicillin biosynthesis genes (Martín, 2000). In this case, alkaline pH values, which strongly stimulate penicillin biosynthesis genes in *Acremonium chrysogenum*, have only a slight effect on the same genes in *Penicillium chrysogenum* (Martín, 2000). It is important to remark that the functionality of the PacC binding sites in xylanase expression has not been directly demonstrated by gel shift assays, reporter gene expression or other suitable techniques. These analyzes will be important to clarify the role of PacC in xylanase expression regulation.

5.4. An integrated view of regulation

In spite of the limited information about xylanolytic gene expression in *Penicillium* (mainly obtained from *P. purpurogenum* studies), it is possible to propose a basic model for the action for these enzymes. As a general rule, all the *Penicillium* xylanases studied are repressed by glucose, and those enzymes containing CBMs can bind to polymeric substrates. On the contrary, enzymes lacking CBM attack soluble molecules. Most of these fungi seem to express their enzymes at neutral pH, although there are exceptions, such as the XynA from *Penicillium* sp. 40.

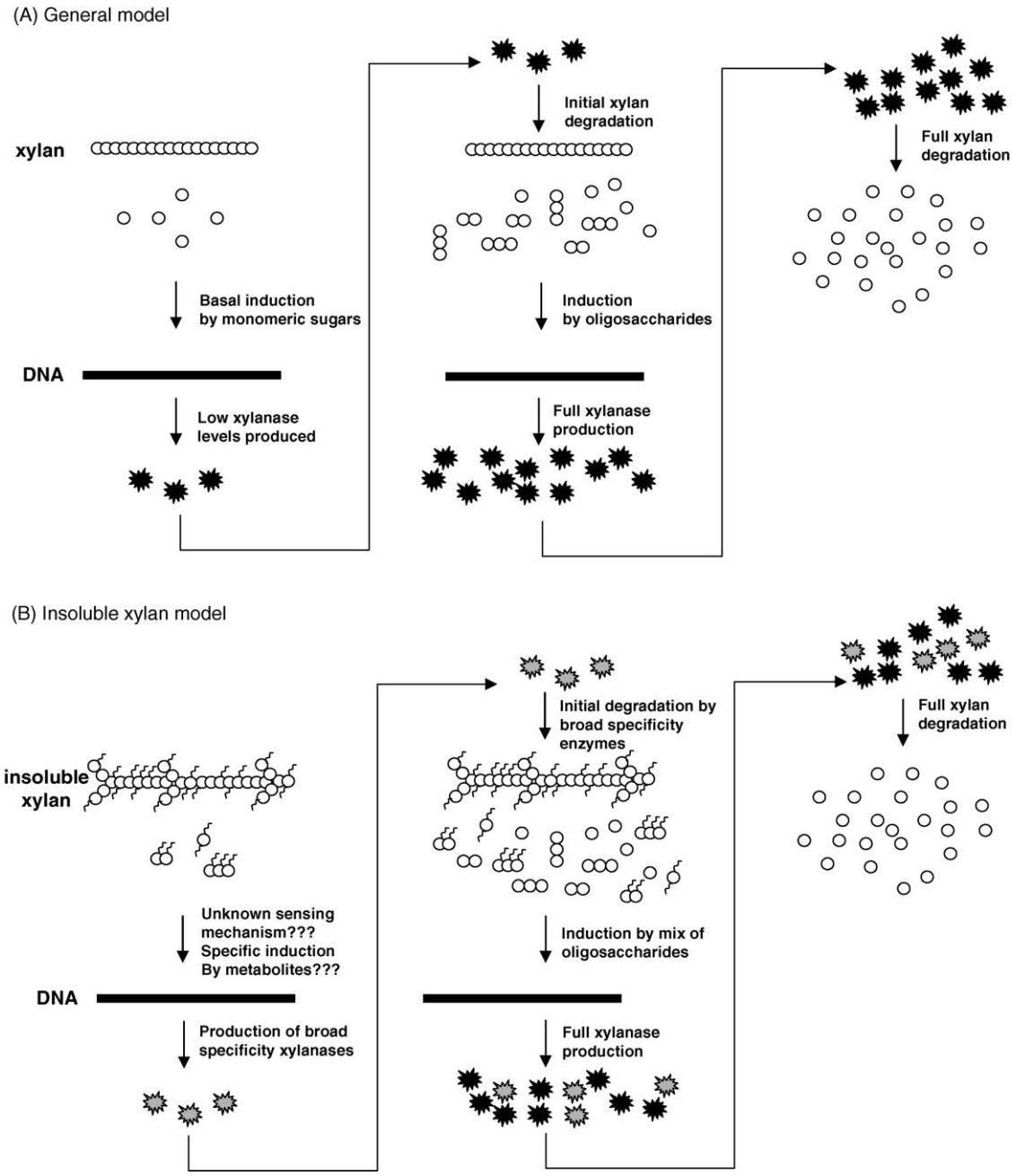

Fig. 3. Proposed model for xylanase expression and xylan degradation by *Penicillium*. (A) Low levels of enzymes (black stars) are induced by few molecules of monomeric sugars such as xylose (white circles). The action of these enzymes on xylan produces oligosaccharides, which induce full xylanase expression, unleashing complete xylan degradation. (B) If xylan is highly insoluble, the fungus senses its presence by an unknown mechanism, and induces broad specificity xylanases (gray stars). These enzymes begin the degradation of this xylan, producing a mixture of oligosaccharides, which induces the full production of xylanases, thus allowing complete xylan degradation. More details are given in the text.

Initially, in the presence of few molecules of monomeric sugars such as xylose or xylitol (produced, for example, by chemical or mechanical breakage of xylan), low levels of expression of some enzymes are detected (Fig. 3A). This basal expression level would be a "stand by" condition for the possible availability of xylan as a main substrate. This proposition is corroborated by the low, but detectable level of transcripts for some xylanase genes in the presence of simple sugars (Zadra et al., 2000; Chávez et al., 2002a; Chávez et al., 2004), and the observations by Jørgensen et al. (2004), who recorded detectable xylanase activities in three *Penicillium* species (*P. pinophilum*, *P. persicinum* and *P. brasilianum*) grown in xylose, thus suggesting that a basal production of xylanases may be achieved in these organisms in the presence of this sugar.

If xylan is available, this basal xylanase activity would digest some of the polysaccharide, releasing oligosaccharides that could enter the cell and trigger full expression of xylanases, thus unleashing the total degradation of xylan (Fig. 3A). This mechanism has some resemblance to the basal expression of cellulolytic enzymes proposed for *T. reesei* (Carle-Urioste et al., 1997).

However, if xylan is particularly insoluble, the basal enzymes may not be efficient for its initial degradation and the release of oligosaccharides needed for the full expression of xylanases. In this case, enzymes with broader degrading ability are necessary. These enzymes are not induced by monomeric sugars or by some types of xylan (such as birchwood xylan), but are induced by other xylan molecules (oat spelt xylan, for example). These enzymes could be induced by the chemical or mechanical release of specific oligosaccharides from xylan, or by some other unknown sensing mechanism (Fig. 3B). Thus, the initial degradation of insoluble xylan by enzymes with broader degradation ability will release the oligosaccharides necessary for the full expression of xylanases. Enzymes with broader degradation ability are endoxylanase A from *P. purpurogenum* or endoxylanase from *P. simplicissimum*. These enzymes, belonging to family 10 of the glycosyl hydrolases, have a broader and shallower active site cleft than other endoxylanases, implying that they would be more accessible to this kind of substrates (Schmidt et al., 1998; Chávez et al., 2001a). In addition, family 10 endoxylanases have low substrate specificity (Biely et al., 1997). Supporting the proposed model, XynA from *P. purpurogenum* is only expressed in the presence of oat spelts xylan, but not birchwood xylan, xylose or xylitol, which are efficient inducers for other xylanases (such as family 11 XynB) from the same organism (Chávez et al., 2002a).

In a comprehensive review about bacterial, yeast and fungal xylanases, Kulkarni et al. (1999) proposed an expression regulation model, which differs to the model described in this work (Fig. 3). Both models suggest that the initial release of low molecular mass metabolites (xylose and oligosaccharides) from xylan plays a key role in full xylanases induction. However, Kulkarni's group hypothesizes that these metabolites are liberated from xylan by the action of small amounts of constitutively produced xylanases. Although not obvious from reading this review, it seems that this proposal is based on a paper cited by the authors, describing a xylanase-producing strain of *Bacillus subtilis* resistant to glucose repression (Srivastava and Srivastava, 1993). To our knowledge, no similar case has been found in *Penicillium* or other fungi. In fact, glucose acts as repressor in all fungal xylanolytic genes where the regulation has been studied at the molecular level (several cases are described in previous sections), and evidence for the constitutive production of xylanase has not been reported. This leads us to discard the "constitutive xylanases hypothesis" in *Penicillium*.

In summary, we propose a model with different levels of complexity for the expression of xylanolytic enzymes, depending on substrate availability (Fig. 3). In principle, there would be a basal level of expression of some enzymes produced by monomeric sugars (xylose or xylitol). This basal level of enzymes, when xylan is the main substrate, would produce oligosaccharides that induce a higher level of xylanolytic activity. In most cases, this set of enzymes would be sufficient for the degradation of several kinds of xylan. If the xylan molecule is too insoluble, enzymes with broader substrate specificity and accessibility would be necessary to obtain oligosaccharides, which would then induce full enzyme production. As we have seen, several aspects about the induction of xylanases by xylan and their derived metabolites in *Penicillium* (and other filamentous fungus) are puzzling, and the use of inducer oligosaccharides of different composition and structure will be necessary to clarify these points.

6. Biotechnological applications

Xylanases are known for a number of biotechnological applications. An important use is in cellulose pulp biobleaching (Polizeli et al., 2005), to eliminate lignin residues from kraft pulp. Xylanases increase the digestibility of feed by lowering the viscosity in the intestinal tract, thus improving nutrient uptake (Twomey et al., 2003). In baking, they are added to increase the specific bread volume and in this way improve final flavor (Maat et al., 1992). In beer and juice processing, xylanases are utilized to reduce haze formation by solubilizing long chain arabinoxylans (Dervilly et al., 2002). Applications have also been found in textile manufacture (Prade, 1995). Another potential use of the xylanases is in the saccharification of xylan, the second most abundant polysaccharide on earth, for its eventual conversion in fuels and chemicals. Important recent advances are discussed by Saha (2000). Thus, there is ample need to find appropriate enzymes for these and other potential biotechnology uses, and xylanase from *Penicillia* may find a place in these applications.

An enzyme from *P. janthinellum* (Milagres and Prade, 1994), a strain which produces no detectable cellulolytic activity, has been used for the recovery of cellulosic textile fibers. The endoxylanase from *Penicillium* sp. 40 (Kimura et al., 2000), which has a highly acidic pH optimum may find application in processes requiring an acid milieu. Bradner et al. (1999) have found significant endoxylanase activity at 10–20 °C produced by Antarctic isolates of *Penicillia*; these enzymes may be useful for low-temperature applications such as improvement in juice color and clarity and in baking (Collins et al., 2005). A xylanolytic complex from *P. canescens* has been used for enzymatic pretreatment of kraft pulp in cellulose biobleaching assays (Curotto et al., 1998), and a recombinant form of this organism's endoxylanase has also been utilized in biobleaching trials (Sinitsyna et al., 2003b).

In recent years, arabinan-degrading enzymes are finding applications, such as in the processing of foods, vegetables and juices, the bioconversion of plant biomass, improvement of animal feedstock digestibility, delignification of cellulose pulp and liberation of aromatic monoterpenes in wine processing (Saha, 2000). The cold-adapted endo arabinanase from *P. chrysogenum* may prove useful in the enzymatic treatment of juice concentrates to prevent quality deterioration (Sakamoto et al., 2003).

Feruloyl esterases show several possible biotechnological applications, particularly in food processing and supplementing endogenous digestive enzymes in animals fed with low-grade foodstuffs, based on their synergistic activities with other xylanases. Ferulic acid is an effective antioxidant, with potential use in the food and pharmaceutical industries; so, these enzymes can be useful for the extraction of hydroxycinnamic acids from natural sources particularly from agricultural waste products (Kroon and Williamson, 1999). The enzymes from *Penicillia* here described may be useful in this respect, depending on their substrate specificity.

A general discussion of the different applications of xylanases can be found in the reviews of Bhat (2000), Beg et al. (2001) and Polizeli et al. (2005).

Acknowledgements

The authors acknowledge the support received from DIPUC, FONDECYT and Universidad Andrés Bello through research grants and fellowships awarded during the course of the work performed in the study of the *Penicillium purpurogenum* xylanolytic enzyme system.

References

Abdel-Sater, M.A., El Said, A.H.M., 2001. Xylan-decomposing fungi and xylanolytic activity in agricultural and industrial wastes. Int. Biodeterior. Biodegrad. 47, 15–21.

Alcocer, M.J.C., Furniss, C.S.M., Kroon, P.A., Campbell, M., Archer, D.B., 2003. Comparison of modular and non-modular xylanases as carrier proteins for the efficient secretion of heterologous proteins from *Penicillium funiculosum*. Appl. Microbiol. Biotechnol. 60, 726–732.

Bakri, Y., Jacques, P., Thonart, P., 2003. Xylanase production by *Penicillium canescens* 10-10c in solid-state fermentation. Appl. Biochem. Biotechnol. 105, 737–748.

Beg, Q.K., Kapoor, M., Mahajan, L., Hoondal, G.S., 2001. Microbial xylanases and their industrial applications: a review. Appl. Microbiol. Biotechnol. 56, 326–338.

Belancic, A., Scarpa, J., Peirano, A., Díaz, R., Steiner, J., Eyzaguirre, J., 1995. *Penicillium purpurogenum* produces several xylanases: purification and properties of two of the enzymes. J. Biotechnol. 41, 71–79.

Beldman, G., Schols, H.A., Pitson, S.M., Searle-van Leeuwn, M.F.J., Voragen, A.G.J., 1997. Arabinans and arabinan degrading enzymes. Adv. Macromol. Carbohydr. Res. 1, 1–64.

Berlin, A.G., Gusakov, A.V., Sinitsyna, O.A., Sinitsyn, A.P., 2000. Detection of major xylanase-containing cellulose-binding domain from *Penicillium verruculosum* by combination of chromatofocusing and limited proteolysis. Appl. Biochem. Biotechnol. 88, 345–352.

Bhat, M.K., 2000. Cellulases and related enzymes in biotechnology. Biotechnol. Adv. 18, 355–383.

Biely, P., Vrsanska, M., Tenkanen, M., Kluepfel, D., 1997. Endo β-1,4-xylanase families: differences in catalytic properties. J. Biotechnol. 57, 151–166.

Biely, P., de Vries, R.P., Vrsanská, M., Visser, J., 2000. Inverting character of α-glucuronidase A from *Aspergillus tubigensis*. Biochim. Biophys. Acta 1474, 360–364.

Bradner, J.R., Sidhu, R.K., Gillings, M., Nevalainen, K.M.H., 1999. Hemicellulase activity of antarctic microfungi. J. Appl. Microbiol. 87, 366–370.

Brown, J.A., Collin, S.A., Wood, T.M., 1987. Development of a medium for high cellulase, xylanase and β-glucosidase production by a mutant strain (NTG III/6) of the cellulolytic fungus *Penicillium pinophilum*. Enzyme Microb. Technol. 9, 355–360.

Carle-Urioste, J.C., Escobar-Vera, J., El-Gogary, S., Enrique-Silva, F., Torigoi, E., Crivellaro, O., Herrera-Estrella, A., El-Dorry, H., 1997. Cellulase induction in *Trichoderma reesei* by cellulose requires its own basal expression. J. Biol. Chem. 272, 10169–10174.

Carter, G.L., Allison, D., Rey, M.W., Dunn-Coleman, N.S., 1992. Chromosomal and genetic analysis of the electrophoretic karyotype of *Trichoderma reesei*: mapping of the cellulase and xylanase genes. Mol. Microbiol. 6, 2174–2617.

Carvallo, M., De Ioannes, P., Navarro, C., Chávez, R., Peirano, A., Bull, P., Eyzaguirre, J., 2003. Characterization of an α-L-arabinofuranosidase gene (*abf1*) from *Penicillium purpurogenum* and its expression. Mycol. Res. 107, 388–394.

Castanares, A., McCrae, S.I., Wood, T.M., 1992. Purification and properties of a feruloyl/*p*-coumaroyl esterase from the fungus *Penicillium pinophilum*. Enzyme Microb. Technol. 14, 875–884.

Chávez, R., Almarza, C., Schachter, K., Peirano, A., Bull, P., Eyzaguirre, J., 2001a. Structure analysis of the endoxylanase A gene from *Penicillium purpurogenum*. Biol. Res. 34, 217–226.

Chávez, R., Fierro, F., Gordillo, F., Martín, J.F., Eyzaguirre, J., 2001b. Electrophoretic karyotype of the filamentous fungus *Penicillium purpurogenum* and chromosomal location of several xylanolytic genes. FEMS Microbiol. Lett. 205, 379–383.

Chávez, R., Schachter, K., Navarro, C., Peirano, A., Aguirre, C., Bull, P., Eyzaguirre, J., 2002a. Differences in expresión of two endoxylanase genes (*xynA* and *xynB*) from *Penicillium purpurogenum*. Gene 293, 161–168.

Chávez, R., Navarro, C., Calderón, I., Peirano, A., Bull, P., Eyzaguirre, J., 2002b. Secretion of endoxylanase A from *Penicillium purpurogenum* by *Saccharomyces cerevisiae* transformed with genomic fungal DNA. FEMS Microbiol. Lett. 212, 237–241.

Chávez, R., Schachter, K., Navarro, C., Peirano, A., Bull, P., Eyzaguirre, J., 2004. The acetyl xylan esterase II gene from *Penicillium purpurogenum* is differentially expressed in several carbon sources, and tightly regulated by pH. Biol. Res. 37, 107–113.

Collins, T., Gerday, C., Feller, G., 2005. Xylanases, xylanase families and extremophilic xylanases. FEMS Microbiol. Rev. 29, 3–23.

Covert, S.F., Vanden Wymelenberg, A., Cullen, D., 1992. Structure, organization, and transcription of a cellobiohydrolase gene cluster from *Phanerochaete chrysosporium*. Appl. Environ. Microbiol. 58, 2168–2175.

Crepin, V.F., Faulds, C.B., Connerton, I.F., 2004. Functional classification of the microbial feruloyl esterases. Appl. Microbiol. Biotechnol. 63, 647–652.

Cubero, B., Scazzocchio, C., 1994. Two different, adjacent and divergent zinc finger binding sites are necessary for CREA-mediated carbon catabolite repression in the proline gene cluster of *Aspergillus nidulans*. EMBO J. 13, 407–415.

Curotto, E., Concha, M., Campos, V., Milagres, A.M.F., Duran, N., 1994. Production of extracellular xylanases by *Penicillium janthinellum*. Appl. Biochem. Biotechnol. 48, 107–116.

Curotto, E., Nazal, A., Aguirre, C., Campos, V., Duran, N., 1998. Enzymatic pretreatment of kraft pulp from *Pinus radiata* D Don with xylanolytic complex of *Penicillium canescens* (CP1) fungi. Appl. Biochem. Biotechnol. 73, 29–42.

De Ioannes, P., Peirano, A., Steiner, J., Eyzaguirre, J., 2000. An α-L-arabinofuranosidase from *Penicillium purpurogenum*: production, purification and properties. J. Biotechnol. 76, 253–258.

Deleyn, F., Claeyssens, M., Van Beeumen, J., De Bruyne, C.K., 1978. Purification and properties of β-xylosidase from *Penicillium wortmanni*. Can. J. Biochem. 56, 43–50.

Denison, S.H., 2000. pH regulation of gene expression in fungi. Fungal Genet. Biol. 29, 61–71.

Depiereux, E., Feytmans, E., 1992. MATCHBOX: a fundamentally new algorithm for the simultaneous alignment of several protein sequences. CABIOS 8, 501–509.

Dervilly, G., Leclercq, C., Zimmermann, D., Roue, C., Thibault, J.-F., Saulnier, L., 2002. Isolation and characterization of high molar mass water-soluble arabinoxylans from barley and barley malt. Carbohydr. Polym. 47, 143–149.

de Vries, R.P., Visser, J., 2001. *Aspergillus* enzymes involved in degradation of plant cell wall polysaccharides. Microbiol. Mol. Biol. Rev. 65, 497–522.

Díaz, R., Sapag, A., Peirano, A., Steiner, J., Eyzaguirre, J., 1997. Cloning, sequencing and expression of the cDNA of endoxylanase B from *Penicillium purpurogenum*. Gene 187, 247–251.

Diez, B., Gutiérrez, S., Barredo, J.L., van Solinger, P., van der Voort, L.H.M., Martín, J.F., 1990. The cluster of penicillin biosynthetic genes. Identification and characterization of the pcbAB gene encoding the α-aminoadipyl-cysteinyl-valine synthetase and linkage to the pcbC and pcbDE genes. J. Biol. Chem. 265, 16358–16365.

Donaghy, J.A., McKay, A.M., 1995. Production of feruloyl/*p*-coumaroyl esterase activity by *Penicillium expansum*, *Penicillium brevicompactum* and *Aspergillus niger*. J. Appl. Bacteriol. 79, 657–662.

Donaghy, J.A., McKay, A.M., 1997. Purification and characterization of a feruloyl esterase from the fungus *Penicillium expansum*. J. Appl. Bacteriol. 83, 718–726.

Egaña, L., Gutiérrez, R., Caputo, V., Peirano, A., Steiner, J., Eyzaguirre, J., 1996. Purification and characterization of two acetyl xylan esterases from *Penicillium purpurogenum*. Biotechnol. Appl. Biochem. 24, 33–39.

Eyzaguirre, J., Scarpa, J., Belancic, A., Steiner, J., 1992. The xylanase system of *Penicillium purpurogenum*. In: Visser, J., Beldman, G., Kusters-van Someren, M.A., Voragen, A.G.J. (Eds.), Xylan and Xylanases. Elsevier Science Publisher B.V, The Netherlands, pp. 505–510.

Fadel, M., Fouda, M.S., 1993. Physiological studies on xylanase production by *Penicillium funiculosum* on some agricultural wastes. Zentralbl. Mikrobiol. 148, 304–312.

Filho, E.X.F., Puls, J., Coughlan, M.P., 1993. Biochemical characteristics of two endo-β-xylanases produced by *Penicillium capsulatum*. J. Ind. Microbiol. 11, 171–180.

Filho, E.X.F., Puls, J., Coughlan, M.P., 1996. Purification and characterization of two arabinofuranosidases from solid-state cultures of the fungus *Penicillium capsulatum*. Appl. Environ. Microbiol. 62, 168–173.

Fillinger, F., Felenbok, B., 1996. A newly identified gene cluster in *Aspergillus nidulans* comprises five novel genes localized in the *alc* region that are controlled both by the specific trans activator AlcR and the general carbon-catabolite repressor CreA. Mol. Microbiol. 20, 475–488.

Funaguma, T., Naito, S., Morita, M., Okumura, M., Sugiura, M., Hara, A., 1991. Purification and some properties of xylanase from *Penicillium herquei* Banier and Sartory. Agric. Biol. Chem. 55, 1163–1165.

Furniss, C.S.M., Belshaw, N.J., Alcocer, M.J.C., Williamson, G., Elliott, G.O., Gebruers, K., Haigh, N.P., Fish, N.M., Kroon, P.A., 2002. A family 11 xylanase from *Penicillium funiculosum* is strongly inhibited by three wheat xylanase inhibitors. Biochim. Biophys. Acta 1598, 24–29.

Furniss, C.S.M., Williamson, G., Kroon, P.A., 2005. The substrate specificity and susceptibility to wheat inhibitor proteins of *Penicillium funiculosum* xylanases from a commercial enzyme preparation. J. Sci. Food. Agric. 85, 574–582.

Galagan, J.E., Calvo, S.E., Borkovich, K.A., Selker, E.U., Read, N.D., Jaffe, D., FitzHugh, W., Ma, L.-J., Smirnov, S., Purcell, S., Rehman, B., Elkins, T., Engels, R., Wang, S., Nielsen, C.B., Butler, J., Endrizzi, M., Qui, D., Ianakiev, P., Pedersen, D., Nelson, M., Washburne, M., Selitrennikoff, C.P., Kinsey, J.A., Braun, E.L., Zelter, A., Schulte, U., Kothe, G.O., Jedd, G., Mewes, W., Staben, C., Marcotte, E., Greenberg, D., Roy, A., Foley, K., Naylor, J., Thomann, N., Barrett, R., Gnerre, S., Kamal, M., Kamvysselis, M., Mauceli, E., Bielke, C., Rudd, S., Frishman, D., Krystofova, S., Rasmussen, C., Metzenberg, R.L., Perkins, D.D., Kroken, S., Cogoni, C., Macino, G., Catcheside, D., Li, W., Pratt, R.J., Osmani, S.A., DeSouza, C.C., Glass, L., Orbach, M.J., Berglund, J., Voelker, R., Yarden, O., Plamann, M., Seiler, S., Dunlap, J., Radford, A., Aramayo, R., Natvig, D.O., Alex, L.A., Mannhaupt, G., Ebbole, D.J., Freitag, M., Paulsen, I., Sachs, M.S., Lander, E.S., Nusbaum, C., Birren, B., 2003. The genome sequence of the filamentous fungus *Neurospora crassa*. Nature 422, 859–868.

Gaskell, J., Dieperink, E., Cullen, D., 1991. Genomic organization of lignin peroxidase genes of *Phanerochaete chrysosporium*. Nucleic Acids Res. 19, 599–603.

Gaspar, A., Cosson, T., Roques, C., Thonart, Ph., 1997. Study on the production of a xylanolytic complex from *Penicillium canescens* 10-10-c. Appl. Biochem. Biotechnol. 76, 45–58.

Gregory, A.C.E., O'Connell, A.P.O., Boldwell, P., 1998. Xylans. Biotechnol. Genet. Eng. Rev. 15, 439–455.

Gutiérrez, R., Cederlund, L., Hjelmqvist, A., Peirano, A., Herrera, F., Ghosh, D., Jörnvall, 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.

Haas, H., Herfurth, E., Stoffler, G., Redl, B., 1992. Purification, characterization and partial amino acid sequences of a xylanase produced by *Penicillium chrysogenum*. Biochim. Biophys. Acta 1117, 279–286.

Hass, H., Friedlin, E., Stöffler, G., Redl, B., 1993. Cloning and structural organization of a xylanase-encoding gene *from Penicillium chrysogenum*. Gene 126, 237–242.

Haltrich, D., Nidetzky, B., Kulbe, K.D., Steiner, W., Zupancic, S., 1996. Production of fungal xylanases. Bioresource Technol. 58, 137–161.

Hamlyn, P.F., Wales, D.S., Sagar, B.F., 1987. Extracellular enzymes of *Penicillium*. In: Peberdy, J.F. (Ed.), *Penicillium* and *Acremonium*. Plenum Press, New York, pp. 245–284.

Henrissat, B., Davies, G., 1997. Structural and sequence-based classification of glycosyl hydrolases. Curr. Opin. Struct. Biol. 7, 637–644.

Ito, T., Yokoyama, E., Sato, H., Ujita, M., Funaguma, T., Furukawa, K., Hara, A., 2003. Xylosidases associated with the cell surface of *Penicilliium herquei* IFO 4674. J. Biosci. Bioeng. 96, 354–359.

Johansson, T., Nyman, P.O., 1996. A cluster of genes encoding major isozymes of lignin peroxidase and manganese peroxidase from the white-rot fungus *Trametes versicolor*. Gene 170, 3–38.

Johnstone, I.L., MaCabe, P.C., Greaves, P., Gurr, S.J., Cole, G.E., Brow, M.A.D., Unkles, S.E., Clutterbuck, A.J., Kinghorn, J.R., Innis, M.A., 1990. Isolation and characterisation of the *crnA-niiA-niaD* gene cluster for nitrate assimilation in *Aspergillus nidulans*. Gene 90, 181–192.

Jørgensen, H., Eriksson, T., Börjesson, J., Tjerneld, F., Olsson, L., 2003. Purification and characterization of five cellulases and one xylanase from *Penicillium brasilianum* IBT [20888]. Enzyme Microb. Technol. 32, 851–861.

Jørgensen, H., Mørkeberg, A., Krogh, K.B.R., Olsson, L., 2004. Growth and enzyme production by three *Penicillium* species on monosaccharides. J. Biotechnol. 109, 295–299.

Jørgensen, H., Mørkeberg, A., Krogh, K.B.R., Olsson, L., 2005. Production of cellulases and hemicellulases by three *Penicillium* species: effect of substrate and evaluation of cellulose adsorption by capillary electrophoresis. Enzyme Microb. Technol. 36, 42–48.

Joseleau, J.P., Comptat, J., Ruel, K., 1992. Chemical structure of xylans and their interaction in the plant cell walls. In: Visser, J., Beldman, G., Kusters-Van Someren, M.A., Voragen, A.G.J. (Eds.), Xylans and Xylanases. Elsevier, Amsterdam, pp. 1–15.

Keller, N.P., Hohn, T.M., 1997. Metabolic pathway gene clusters in filamentous fungi. Fungal Genet. Biol. 21, 17–29.

Kimura, T., Ito, J., Kawano, A., Making, T., Kondo, H., Karita, S., Sakka, K., Ohmiya, K., 2000. Purification, characterization and molecular cloning of acidophilic xylanase from *Penicillium* sp.40. Biosci. Biotechnol. Biochem. 64, 1230–1237.

Kormelink, F.J.M., Voragen, G.J., 1993. Degradation of different [(glucurono)arabino] xylans by a combination of purified xylan-degrading enzymes. Appl. Microbiol. Biotechnol. 38, 688–695.

Krogh, K.B.R., Mørkeberg, A., Jørgensen, H., Frisvad, J.H.C., Olsson, L., 2004. Screening genus *Penicillium* for producers of cellulolytic and xylanolytic enzymes. Appl. Biochem. Biotechnol. 114, 389–401.

Kroon, P.A., Williamson, G., 1999. Hydroxycinnamates in plants and food: current and future perspectives. J. Sci. Food Agric. 79, 355–361.

Kroon, P.A., Williamson, G., Fish, N.M., Archer, D.B., Belshaw, N.J., 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.

Kulkarni, N., Shendye, A., Rao, M., 1999. Molecular and biotechnological aspects of xylanases. FEMS Microbiol. Rev. 23, 411–456.

Laich, F., Fierro, F., Martin, J.F., 2002. Production of penicillin by fungi growing on food products: identification of a complete penicillin gene cluster in *Penicillium griseofulvum* and a truncated cluster in *Penicillium verrucosum*. Appl. Environ. Microbiol. 68, 1211–1219.

Maat, J., Roza, M., Verbakel, J., Stam, H., Santos da Silva, M.J., Bosse, M., Egmond, M.R., Hagemans, M.L.D., van Gorcom, R.F.M., Hessing, J.G.M., van den Hondel, C.A.M.J.J., van Rotterdam, C., 1992. Xylanases and their application in bakery. In: Visser, J., Beldman, G., Kusters-van Someren, M.A., Voragen, A.G.J. (Eds.), Xylans and Xylanases. Elsevier, Amsterdam, pp. 349–360.

Mach, R.L., Strauss, J., Zeilinger, S., Schindler, M., Kubicek, C.P., 1996. Carbon catabolite repression of xylanase I (*xyn1*) gene expression in *Trichoderma reesei*. Mol. Microbiol. 21, 1273–1281.

Mantyla, A.L., Rossi, K.H., Vanhanen, S.A., Penttila, M.E., Suominen, P.L., Nevalainen, K.M., 1992. Electrophoretic karyotyping of wild-type and mutant *Trichoderma longibrachiatum* (*reesei*) strains. Curr. Genet. 21, 471–477.

Martín, J.F., 2000. Molecular control of expression of penicillin biosynthesis genes in fungi: regulatory proteins interact with a bi-directional promoter region. J. Bacteriol. 182, 2355–2362.

Medeiros, R.G., Hanada, R., Filho, E.X.F., 2003. Production of xylan-degrading enzymes from Amazon forest fungal species. Int. Biodeterior. Biodegrad. 52, 97–100.

Milagres, A.M.F., Lacis, L.S., Prade, R.A., 1993. Characterization of xylanase production by a local isolate of *Penicillium janthinellum*. Enzyme Microb. Technol. 15, 248–253.

Milagres, A.M.F., Prade, R.A., 1994. Production of xylanases from *Penicillium janthinellum* and its use in the recovery of cellulosic textile fibers. Enzyme Microb. Technol. 16, 627–632.

Mishra, C., Seeta, R., Rao, M., 1985. Production of xylanolytic enzymes in association with the cellulolytic activities of *Penicillium funiculosum*. Enzyme Microb. Technol. 7, 295–299.

Moreau, A., Roherge, M., Manin, C., Shareck, F., Kuepfel, D., Morosoli, R., 1994. Identification of two acidic residues involved in the catalysis of xylanase A from *Streptomyces lividans*. Biochem. J. 302, 291–295.

Moss, M.O., 1987. Morphology and physiology of *Penicillium* and *Acremoium*. In: Peberdy, J.F. (Ed.), Penicillium and Acremonium. Plenum Press, New York, pp. 37–71.

Orejas, M., MacCabe, A.P., Pérez-González, J.A., Kumar, S., Ramón, D., 1999. Carbon catabolite repression of the *Aspergillus nidulans xlnA* gene. Mol. Microbiol. 31, 177–184.

Polizeli, M.L.T.M., Rizzatti, A.C.S., Monti, R., Terenzi, H.F., Jorge, J.A., Amorim, D.S., 2005. Xylanases from fungi: properties and industrial applications. Appl. Microbiol. Biotechnol. 67, 577–591.

Prade, R.A., 1995. Xylanases: from biology to biotechnology. Biotechnol. Genet. Eng. Rev. 13, 103–131.

Rahman, A.K.M.S., Sugitani, N., Hatsu, M., Takamizawa, K., 2003. A role of xylanase, α-L-arabinofuranosidase, and xylosidase in xylan degradation. Can. J. Microbiol. 49, 58–64.

Rao, M., Gaikwad, S., Mishra, C., Deshpande, V., 1988. Induction and catabolite repression of cellulase in *Penicillium funiculosum*. Appl. Biochem. Biotechnol. 19, 129–137.

Reese, E.T.A., Maguire, A., Parrish, F.W., 1973. Production of β-D-xylopyranosidase by fungi. Can. J. Microbiol. 19, 1065–1074.

Ryan, S.E., Nolan, K., Thompson, R., Gubitz, G.M., Savage, A.V., Tuohy, M.G., 2003. Purification and characterization of a new low molecular weight endoxylanase form *Penicillium capsulatum*. Enzyme Microb. Technol. 33, 775–785.

Saha, B.C., 2000. α-L-Arabinofuranosidases: biochemistry, molecular biology and application in biotechnology. Biotechnol. Adv. 18, 403–423.

Sakamoto, T., Thibault, J.-F., 2001. Exo-arabinanase of *Penicillium chrysogenum* able to release arabinobiose from α-1,5-L-arabinan. Appl. Environ. Microbiol. 67, 3319–3321.

Sakamoto, T., Kawasaki, H., 2003. Purification and properties of two type-B α-L-arabinofuranosidases produced by *Penicillium chrysogenum*. Biochim. Biophys. Acta 1621, 204–210.

Sakamoto, T., Ihara, H., Kozaki, S., Kawasaki, H., 2003. A cold-adapted endo-arabinanase from *Penicillium chrysogenum*. Biochim. Biophys. Acta 1624, 70–75.

Sakamoto, T., Ihara, H., Shibano, A., Kasai, N., Inui, H., y Kawasaki, H., 2004. Molecular characterization of a *Penicillium chrysogenum* exo-1,5-α-L-arabinanase that is structurally distinct from others arabinan-degrading enzymes. FEBS Lett. 560, 199–204.

Schmidt, A., Schlacher, A., Steiner, W., Schwab, H., Kratky, C., 1998. Structure of the xylanase *from Penicillium chrysogenum*. Protein Sci. 7, 2081–2088.

Serebryanyi, V.A., Vavilova, E.A., Chulkin, A.M., Vinetskii, Y.P., 2002. Cloning of *Penicillium canescens* endo-1,4-β-xylanase gene and construction of multicopy strains. Appl. Biochem. Microbiol. 38, 420–426.

Sinitsyna, O.A., Bukhtoyarov, F.E., Gusakov, A.V., Okunev, O.N., Bekkarevitch, A.O., Vinetsky, Y.P., Sinitsyn, A.P., 2003a. Isolation and properties of major components of Penicillium canescens extracellular enzyme complex. Biochemistry (Moscow) 68, 1200–1209.

Sinitsyna, O.A., Gusakov, A.V., Okunev, O.N., Serebryany, V.A., Vavilova, E.A., Vinetsky, Y.P., Sinitsyn, A.P., 2003b. Recombi-

nant endo-β-1,4-xylanase from *Penicillium canescens*. Biochemistry (Moscow) 68, 1313–1319.

Srivastava, R., Srivastava, A.K., 1993. Characterization of a bacterial xylanase resistant to repression by glucose and xylose. Biotechnol. Lett. 15, 847–852.

Steiner, J., Socha, C., Eyzaguirre, J., 1994. Culture conditions for enhanced cellulase production by a native strain of *Penicillium purpurogenum*. World J. Microbiol. Biotechnol. 10, 280–284.

Stewart, P., Cullen, D., 1999. Organization and differential expression of a cluster of lignin peroxidase genes of *Phanerochaete chrysosporium*. J. Bacteriol. 181, 3427–3432.

Sunna, A., Antranikian, G., 1997. Xylanolytic enzymes from fungi and bacteria. Crit. Rev. Biotechnol. 17, 39–67.

Takenishi, S., Tsujisaka, Y., 1973. *Penicillium janthinellum* biourge xylanase. J. Ferment. Technol. 51, 458–463.

Thygesen, A., Thomsen, A.B., Schmidt, A.S., Jørgensen, H., Ahring, B.K., Olsson, L., 2003. Production of cellulose and hemicellulose-degrading enzymes by filamentous fungi cultivated on wet-oxidised wheat straw. Enzyme Microb. Technol. 32, 606–615.

Tilburn, J., Sarkar, S., Widdick, D.A., Espeso, E.A., Orejas, M., Mungroo, J., Peñalva, M.A., Arst Jr., H.N., 1995. The *Aspergillus* PacC zinc-finger transcription factor mediates regulation of both acid- and alkaline-expressed genes by the ambient pH. EMBO J. 14, 779–790.

Twomey, L.N., Pluske, J.R., Rowe, J.B., Choct, M., Brown, W., McConnell, M.F., Pethick, D.W., 2003. The effects of increasing levels of soluble non-starch polysaccharides and inclusion of feed enzymes in dog diets on faecal quality and digestibility. Anim. Feed Sci. Technol. 108, 71–82.

van Peij, N.N.M., Visser, J., de Graaff, L.H., 1998. Isolation and analysis of *xlnR*, encoding a transcriptional activator co-ordinating xylanolytic expression in *Aspergillus niger*. Mol. Microbiol. 27, 131–142.

Wegener, S., Ransom, R.F., Walton, J.D., 1999. A unique eukaryotic β-xylosidase gene from the phytopathogenic fungus *Cochliobolus carbonum*. Microbiology 145, 1089–1095.

Wong, K.K.Y., Saddler, J.N., 1992. *Trichoderma* xylanases, their properties and application. Crit. Rev. Biotechnol. 12, 413–435.

Xu, J., Nogawa, M., Okada, H., Morikawa, Y., 2000. Regulation of *xyn3* gene expresion in *Trichoderma reesei* PC-3-7. Appl. Microbiol. Biotechnol. 54, 370–375.

Zadra, I., Abt, B., Parson, W., Haas, H., 2000. xylP promoter-based expression system and its use for antisense downregulation of the *Penicillium chrysogenum* nitrogen regulator NRE. Appl. Environ. Microbiol. 66, 4810–4816.

Zeilinger, S., Mach, R.L., Schindler, M., Herzog, P., Kubicek, C.P., 1996. Different inducibility of expression of the two xylanases genes *xyn1* and *xyn2* in *Trichoderma reesei*. J. Biol. Chem. 271, 25614–25629.