REVIEW

Cellulases from *Penicillium* species for producing fuels from biomass

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This review focuses on cellulases from *Penicillium* species with emphasis on cellulose biodegradation; the most laborious step in the production of biofuels from lignocellulosic biomass. Extracellular multienzyme systems secreted by various *Penicillium* species proved to be highly efficient destroyers of cellulose in comparison with competitive producers of cellulases. Data on cellulase production levels by *Penicillia*, enzyme induction and regulation, composition of the secreted multienzyme systems, examples of their application for hydrolysis of lignocellulosic residues, as well as reasons for their high saccharification performance, are discussed. Analysis of the data shows that *Penicillium* cellulases are very promising candidates for production of the second-generation biofuels; however, some issues should be resolved in order for them to become cost competitive to enzymes produced by other microorganisms (in particular, to those from *Trichoderma reesei*).

Second-generation biofuels, such as ethanol, butanol, mixed alcohols or various oils derived from non-food **plant lignocellulosic biomass**, are a hot topic due to the limited fossil resources and increases in oil prices. Every year hundreds, if not thousands, of articles and reports are published, and many international and local conferences are organized, on the subject of second-generation biofuels in different countries. The most recent information concerning various aspects of second-generation biofuels may be found in reviews [1–4]. There are two major approaches for producing liquid biofuels from biomass: thermochemical and biochemical processing. Both routes have advantages and drawbacks. The biochemical processing, in which enzymes are used to convert cellulose and hemicellulose components of the biomass to sugars prior to their fermentation to ethanol or other liquid fuels, takes place under milder conditions and it is more environmentally friendly. However the main obstacle in this route is the recalcitrant crystalline nature of cellulosic fibers that makes the enzymatic hydrolysis of cellulose time and enzyme consuming. So, a pretreatment of the feedstock is necessary to provide faster and more complete conversion of cellulose during its enzymatic hydrolysis [5]. Another crucial point in creating more effective and economically viable technology of the lignocellulosic feedstock conversion to liquid fuels is connected with enzymes hydrolyzing biomass components. Finding more active cellulases and their producers, optimization of the multienzyme cocktail for cellulose and hemicellulose hydrolysis, expression of the optimal multienzyme composition at high level of the protein biosynthesis and reducing the cost of enzyme production are the most important tasks herein [6–10].

The enzymatic hydrolysis of cellulose occurs under the synergistic action of endo-1,4-β-glucanases (EGs) and exo-cellobiohydrolases (CBHs) yielding cellobiose as a major product, together with other cello-oligosaccharides and glucose [6], with oligosaccharides finally converted to glucose by β-glucosidases (BGLs). Hemicelluloses, represented mainly as xylans, galactoglucomannans and xyloglucans in the lignocellulosic biomass, are hydrolyzed by a system of hemicellulase enzymes: endo-1,4-β-xylanases, β-xylosidases, α-L-arabinofuranosidases, α-D-glucuronidases, acetylxylan esterases, feruloyl and coumaroyl esterases, endo-1,4-β-mannanases, β-mannosidases, α-galactosidases,

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Key terms

Plant lignocellulosic biomass: Agricultural and forest residues, grasses, aquatic biomass, the major components of which are cellulose, hemicelluloses and lignin. The first two components are polysaccharides that may be converted to fermentable sugars by enzymatic hydrolysis.

Filamentous fungi: Mold fungi that grow in the form of multicellular fragments called hyphae. They secrete extracellular hydrolytic enzymes that degrade the components of plant biomass (cellulose and other polysaccharides) into smaller fragments and monomer sugars.

Submerged fermentation: Cellulases and hemicellulases are produced by filamentous fungi using submerged fermentations in liquid media containing a carbon source (substrate) and nutritional components. At a laboratory scale, the fermentations are often carried out in shake flasks, while at an industry scale they are carried out in big tanks (fermenters). Sometimes fungi are grown on a solid substrate; this process is called solid state fermentation.

Cellulase activity: Enzyme activity toward Whatman filter paper No.1 (filter paper activity) is a standard assay of cellulase activity accepted in most laboratories worldwide. The CMCase activity assay against a soluble derivative of cellulose, carboxymethylcellulose, is also widely used by researchers. The β-glucosidase activity is usually measured against cellobiose or synthetic nitrophenyl derivative of glucose (o-NP or p-NP-β-D-glucopyranoside).

acetyl mannan esterases and xyloglucanases [11]. Hemicellulases also act as accessory enzymes to cellulases, providing more efficient conversion of cellulose [12].

Filamentous fungi are the major source of commercial cellulases and hemicellulases, usually produced by **submerged fermentation**. Mutant strains of *Trichoderma reesei* are considered indisputable champions in cellulase production and enzyme activity among the biomass-degrading fungi [6,8,13]. Therfore, it is not surprising that most of R&D projects on bioethanol production from lignocellulosics have been based on *T. reesei* cellulases [7,8]. However, recent publications have increasingly demonstrated that alternatives to *T. reesei* enzymes in the production of second-generation biofuels exist; in particular, cellulases secreted by various *Penicillium* species provided higher cellulose conversions and glucose yields than those from *T. reesei* at the same **cellulase activity** or protein loading in the reaction system [13].

Penicillium belongs phylogenetically to the Trichocomaceae family and more than 250 species are currently accepted in this genus [14]. With the exception of *Penicillium marneffei*, *Penicillia* are filamentous fungi. *Penicillium* species are widely present in the air and dust of indoor environments; a few of them even play a central role in the production of cheese and various meat products. *Penicillia* are the source of major antibiotics, including penicillin, discovered by Alexander Fleming in 1928. Various *Penicillium* mutant strains are used for industrial enzyme production (e.g., lipase, pectinase and glucose oxidase) [15]. Although some of the *Penicillium* species have long been known as a source of cellulases and hemicellulases [16–18], their industrial production is not widespread. One example is Rovabio® Excel, a commercial *P. funiculosum* enzyme product, possessing high cellulase and xylanase activities, from the Adisseo (France) company [301]. Cellulases and hemicellulases from *Penicillium* species are a rather popular subject of research, although they can not compete with the popularity of *Trichoderma* enzymes. A recent search in the Web of Science database with the keywords 'cellulase AND Penicillium' in the topic of papers from 2001 to present resulted in 200 documents, while for 'cellulase AND Trichoderma' the result was 1448 hits [302]. For comparison, in the case of *Aspergilli*, other well-known fungal producers of industrial enzymes, the search with 'cellulase AND Aspergillus' keywords gave the result of 719 papers for the same timespan. Regarding hemicellulases, a comprehensive review by Chávez *et al.* provided extensive information on xylanolytic enzyme systems from the fungi belonging to the genus *Penicillium* [16].

This review paper focuses on cellulases from *Penicillium* species with emphasis on cellulose biodegradation, the most laborious step in the production of biofuels from lignocellulosic biomass. The following topics will be discussed in this paper:

- Production of cellulases by *Penicillia*;
- Enzyme induction and regulation;
- Cellulase system of *Penicillium* fungi and its relation to enzyme systems of other fungi;
- Saccharification of different lignocellulosic residues using cellulases from *Penicillium* species;
- Specific features responsible for high hydrolytic efficiency of cellulase systems secreted by *Penicillia*.

Cellulase production by *Penicillium* species

Penicillia secrete extracellular enzymes of different specificity, such as lipases, cellulases, xylanases, pectinases and proteases [17]. Some species are characterized by enhanced production of cellulases; among them are: *P. brasilianum*, *P. brevicompactum*, *P. citrinum*, *P. chrysogenum*, *P. crustossum*, *P. decumbens*, *P. echinulatum*, *P. expansum*, *P. funiculosum*, *P. glabrum*, *P. griseoroseum*, *P. janthinellum*, *P. minioluteum*, *P. occitanis*, *P. persicinum*, *P. pinophilum*, *P. purpurogenum* and *P. verruculosum* [18–37].

Data on the production of cellulases by various *Penicillium* species are summarized in **Table 1**. In most cases listed, the submerged fermentations were carried out (either in shake flasks or laboratory-scale fermenters) [18,19,21–24,27,29,31,33–37]. The level of the filter paper activity (FPA) attained in shake flasks (**Table 1**, upper part, except for the last three rows where data for laboratory fermenters are shown) varied in the range of 0.3–3.5 filter paper unit (FPU)/ml; the protein concentration, enzyme action against carboxymethylcellulose (CMCase) and BGL activity was in the range of 0.27–2.22 g/l, 2–98 and 0.5–8 U/ml, respectively. In a few cases reported, solid state fermentations of *Penicillium* fungi have been carried out [30,32,36]. The FPA and BGL activity obtained varied in the range of 6–68 FPU/g substrate and 59–149 U/g substrate. It is difficult to compare these results with those obtained for submerged fermentations; however,

Table 1. Cellulase production by *Penicillium* species[†].

Organism	Substrate	Fermentation time (h)	Protein (g/l)	FPA (FPU/ml)	CMCase (U/ml)	BGL (U/ml)	Ref.
Penicillium minioluteum IBT 21486	Solka-Floc cellulose (2%)	230		0.29	9	1.70	[21]
P. pinophilum IBT 10872				0.32	6	2.45	
P. verruculosum IBT 18366				0.37	12	0.97	
P. brasilianum IBT 20888				0.68	98	1.09	
P. funiculosum ATCC11797	CMC, Avicel, sugarcane bagasse derived materials (7.5 g/l)	81–276		0.35	3.59	1.84	[35]
P. brasilianum IBT 20888	Sigmacell 20 cellulose (20 g/l), pretreated spruce (35.8 g/l)	165	0.5	0.59	19	3.5	[31]
P. brasilianum IBT 20888	Solka-Floc 200 FCC, oat spelts xylan, birchwood xylan (40 g/l)	170	1.07	0.75			[22]
P. pinophilum IBT 4186			1.08	0.81			
P. persicinum IBT 13226			2.22	1.7			
P. decumbens 114–2	Wheat bran		0.36	0.83	5.4	2.39	[29]
P. decumbens JU-A10			0.51	1.96	10.6	1.02	
P. echinulatum 9A02S1	Cellulose plus lactose (1% in total)	168	0.27	1.6		0.5	[24]
P. citrinum MTCC 6489	Wheat bran	72–168	0.65	1.72	1.89		[27]
P. echinulatum S1M29	Microcrystalline cellulose (1%) plus soy bran (0.2%) plus glucose or sucrose (0.5–1%)	144		2.0	17	1.5	[34]
P. funiculosum	Cellulose (2%) plus cellobiose (0.5 mg/l)	240		2.5	7	8	[18]
P. echinulatum 9A02S1	Cellulose (1%) plus glucose (0–1.5%) plus methylxanthines (1–5 µM)	192		3.1		2.9	[33]
P. janthinellum EU2D-21	Avicel, Solka-Floc SW44, cellulose or tissue paper (1%) plus wheat bran (2.5%)[‡]	192 or 96		3.49 67.8[‡]	94.4 3558[‡]	3.2 149[‡]	[36]
P. decumbens ML-017	Rice bran	72		5.76[‡]			[30]
P. echinulatum 9A02S1	Pretreated sugarcane bagasse, wheat bran	120		33[‡]	282[‡]	59[‡]	[32]
P. pinophilum NTG III/6	Solka-Floc BW40, milled barley straw, wheat bran, Avicel PH101 (1–6%)	72–240	14.5	9.8	175	38	[37]
P. occitanis Pol6	Avicel PH101 (8%)	187	11.4	12.6	12	17.5	[19]
P. verruculosum (various strains)	Microcrystalline cellulose, fed-batch mode with glucose	144	47		496	61	[23]

[†]The highest activity values attained are listed.
[‡]Activities obtained in solid-state fermentation; expressed in U/g substrate.
BGL: β-glucosidase; CMC: Carboxymethylcellulose; FPA: Filter paper activity; FPU: Filter paper unit.

it should be noted that for *T. reesei* the cellulase yields of 200 FPU/g cellulose are rather typical [6,38].

Krogh *et al.* [21] carried out screening of the *Penicillium* genus for cellulase and xylanase activity, and compared activities obtained with data for *T. reesei* Rut C30 strain, a reference strain among *T. reesei* high cellulase producers. Four *Penicillium* species with enhanced cellulase production have been found, for which FPA in shake flasks varied from 0.29 to 0.68 FPU/ml, while the BGL activity varied in the range of 0.97–2.45 U/ml (**Table 1**; upper part). The *T. reesei* Rut C30 produced 0.54 FPU/ml and only 0.03 U/ml of BGL under the same conditions [21]. According to the data of other researchers, various *T. reesei* mutant strains, including Rut C30, produced 1.2–1.3 FPU/ml during shake flask cultivation on 1% Sigmacell cellulose; the protein concentration, CMCase and BGL activity varied in the range of 1.7–2.14 g/l, 2.96–3.35 U/ml and 0.1–0.6 U/ml, respectively [38]. It should be noted that the production of cellulase protein by *T. reesei* dramatically increased at a higher substrate concentration and after the transition of fermentations from shake flasks to laboratory-and pilot-scale fermenters. Thus, up to 57 FPU/ml and 22 g/l of protein were obtained for various *T. reesei* strains cultivated on 2–15% cellulosic substrates of different origin in fermenters operating in batch or fed batch mode [38]. Even higher

Key term

Glycoside hydrolase families: Cellulases belong to a widespread group of enzymes called glycoside hydrolases. Based on amino acid sequence similarities, the glycoside hydrolases are classified in different protein families. Within one family the enzymes display common fold, similar structure of the active site and the same stereochemistry of the reaction catalyzed. There were 130 glycoside hydrolase families at the beginning of 2012.

numbers have been reported for industrial fermentations of *T. reesei*, up to 75 FPU/ml and 100 g/l of extracellular protein [38,39].

As in the case of *T. reesei*, when *P. pinophilum*, *P. occitanis* and *P. verruculosum* were cultured in laboratory fermenters (Table 1; last three rows), cellulase and BGL activities attained were much higher than those produced by *Penicillium* species in shake flasks. Up to 14.5 g/l of protein, 9.8 FPU/ml and 38 U/ml of BGL were achieved in submerged culture of *P. pinophilum* NTG III/6 in a 16–1 stirred-tank fermenter. The cellulase productivity of *P. pinophilum* NTG III/6 was comparable to that of *T. reesei* Rut C30 strain [37]. Similar levels of protein production (11.4 g/l) and FPA (12.6 FPU/ml) were attained for *P. occitanis* Pol6 grown on 8% microcrystalline cellulose in a 2-l fermenter [19]. *P. verruculosum* secreted up to 47 g/l of protein in a 7-l fermenter operating in fed batch mode [23]. The CMCase and BGL activities attained by *P. verruculosum* were also dramatically higher than those reported for *Penicillium* species by other researchers (Table 1). The protein concentrations obtained in *P. verruculosum* fermentations are on a level with the highest standards for protein production by fungi [23].

In all cases listed in Table 1, *Penicillium* species secreted high levels of the BGL. The ratio of BGL:FPA activity was greater than one in many cases or, at least, the activity numbers were comparable in magnitude. A high level of the BGL activity is necessary to provide fast and complete conversion of cellobiose to glucose. Cellobiose is the intermediate product of enzymatic cellulose hydrolysis; being inhibitory to CBHs, this disaccharide reduces the overall conversion of cellulose to the final product [6–9,12,13]. Compared to *Penicillia*, various strains of *T. reesei* are usually characterized by essentially lower secretion of BGL (the ratio of BGL:FPA notably less than one), since most of BGLs found in the genome of the latter fungus are intracellular [6,8,39]. Thus, the *T. reesei* cellulase preparations had to be supplemented with additional BGL (usually from *Aspergillus* sp.) [8,9,13]. However, the situation with BGL production by *T. reesei* has been improved recently owing to heterologously expressed BGLs from other microorganisms [8,13].

Enzyme induction & regulation

The mechanisms of induction and regulation of cellulase biosynthesis have not been studied as thoroughly in *Penicillia* as in the *Trichoderma* and *Aspergillus* genera. Cellulases are induced in fungi only in the presence of cellulose or other inducers, such as different oligosaccharides (e.g., sophorose, cellobiose and gentiobiose). Different kinds of pure cellulose or cellulose-containing residues were found to be good inducers and substrates for growth and cellulase production by *Penicillium* species (Table 1) [18,21,22,31]. Significant differences were found in the enzyme induction and regulation for *Trichoderma* and *Penicillium* fungi. Sophorose is known to be an effective inducer of cellulases in *Trichoderma,* while not being an inducer in *P. janthinellum* or *P. purpurogenum* [22,40]. The cellulase induction by sophorose seems to be largely specific for *T. reesei*. Cellobiose (1 mg/l) and gentiobiose (0.5 g/l) induced the formation of cellulases in *P. funiculosum* [18] and *P. purpurogenum* [40]. On the contrary, cellobiose was found to be a repressor of the transcription of six major cellulase and xylanase genes in *P. decumbens* [41]. Lactose (5–10 g/l) had no effect on the synthesis of cellulase in *P. funiculosum* or *P. echinulatum* (FPA or CMCase activity), but did induce BGL [18,24]. Cellulase induction in *P. purpurogenum* by cellobiose-octaacetate and other acetyl cellobioses has also been described [42].

The induction of both hemicellulolytic and cellulolytic enzyme systems through the XlnR transcriptional activator has been reported for *Aspergilli* [43,44]. The *Aspergillus niger* XlnR gene was incorporated into the *P. canescens* genome [45]. Integration of the XlnR gene resulted in the increase in a number of activities responsible for xylan degradation; however, cellulase (CMCase) and BGL activities remained constant, compared with the host *P. canescens* PCA 10 strain. Data of Krogh *et al.* [46] provided evidence that the expression of the cellulolytic system in *P. brasilianum* can be mediated by an XlnR-like activator as found in *Aspergilli.* Putative XlnR binding sites have also been reported for xylanolytic enzymes in *P. citrinum* [47]. Further studies should be done to confirm the role of the XlnR transcriptional activator in expression of cellulases in *Penicillia*.

The final product of cellulose hydrolysis (glucose) repressed cellulase and xylanase biosynthesis in different *Penicillium* species as well as exerting repression on the catabolism of other monosaccharides (e.g., mannose, galactose, xylose and arabinose) [41,48]. Obtaining derepressed fungal mutant strains is very important in order to construct industrial cellulase hyperproducers [40,49]. The first generation of catabolite-derepressed mutants of *T. reesei*, including Rut C30, have been obtained by random mutagenesis using UV light or *N*-nitroguanidine [49]. Using a similar approach, the derepressed mutant JU-A10 of *P. decumbens* has been obtained [41]. The genetic basis for the catabolite derepression exhibited by *T. reesei* mutant strains became apparent when a glucose repressor gene, *cre1*, was isolated; this gene was found to be truncated in the Rut C30 strain [49]. Some modern derepressed mutant strains of *T. reesei* have been

constructed by either completely removing the *cre1* gene or replacing it with its truncated version from Rut C30 [49]. The CreA proteins regulate carbon catabolite repression also in *Aspergilli* and other fungi [40]. The presence of the CreA transcriptional regulator was recently demonstrated in *P. janthinellum* [46] and *P. canescens* [50]. Therefore, it seems that the mechanism of carbon catabolite repression is similar in different fungi; the CreA (Cre1) protein being the major regulating factor.

Cellulolytic system of *Penicillium* fungi

Cellulases and BGLs have been purified from *Penicillia* since the 1980s. However, in these earlier papers, the isolated enzymes have been classified only from the point of view of their substrate specificity, without obtaining information on their amino acid sequences and the genes encoding them [51–58]. Therefore, although earlier information provided some classic insights into the mechanism of enzymatic cellulose hydrolysis and cellulase function, it is often difficult to correlate the data with more recent information on *Penicillium* enzymes, classified according to their affiliation into **glycoside hydrolase (GH) families** [59]; this classification has become widely accepted in the last 20 years and is permanently available through the carbohydrate-active enzyme (CAZy) database [303]. As such, we will focus our discussion on more recent data [25,46,60–76].

Information on *Penicillium* cellulases and BGLs, whose complete or partial amino acid sequences are known, is summarized in **Table 2**. The cellulose-degrading multienzyme system of *Penicillium* species is rather typical for fungi. It consists of a few endoglucanases belonging to the GH families 5, 6, 7, 12 and 45, two or more GH6 and GH7 family CBHs (CBH II and CBH I, respectively), and GH1 and GH3 BGLs **(Table 2)**. The most abundant *Penicillium* enzymes in the CAZy database are those from *P. chrysogenum* Wisconsin 54–1255 strain, for which the full fungal genome has recently been sequenced [60]. However, their properties have not been studied and the enzymes are not classified according to their substrate specificity (as EG, CBH or BGL); their affiliation into a particular family is based on amino acid sequences translated from the respective genes. Although Houbraken *et al.* [77] recently demonstrated that both Fleming's penicillin producing strain and Wisconsin 54–1255 strain are not *P. chrysogenum* but *P. rubens*, we will conserve the name *P. chrysogenum* in this review, since it is still present in protein databases and the new *P. rubens* name is not yet widely accepted.

Two families, GH5 represented by endoglucanases, and GH7 represented by CBHs and a few endoglucanases, contain most of *Penicillium* cellulases, for which the sequence information is available **(Table 2)**. Twenty three out of 27 characterized or putative *Penicillium* BGL belong to the GH3 family. So far, only one EG (from *P. decumbens*), with unusually high activity toward glucomannan, belongs to the GH45 family that contains exclusively endoglucanases [73]. Four proteins of the GH61 family were found in the *P. chrysogenum* genome [60]. The GH61 proteins have formerly been classified as endoglucanases; however, very recent studies revealed that they are metal-dependent oxidative enzymes that cleave cellulose [78,79]. These enzymes may act as enhancers of activity of other cellulases [80,81].

Most *Penicillium* CBHs and endoglucanases have a modular structure typical for fungal cellulases; that is, they consist of a catalytic module and a cellulose-binding module (CBM) connected with a flexible peptide linker. However, *Penicillium* cellulases without a CBM, consisting only of a catalytic module, have also been described; for example, GH12 family EGa from *P. brasilianum* [64], GH5 family EG IIa and EG IIb, as well as GH12 EG III from *P. verruculosum* [68]. The single-module structure of these enzymes seems to be encoded on a gene level. However, the low-molecular-weight forms of catalytically active cellulases without a CBM may also be formed as a result of partial proteolysis of the intact enzymes possessing the CBM. Such a situation was observed for EG I as well as for CBH I and II of *P. verruculosum*; high- and low-molecular-weight forms of both CBHs being present in the fungal culture broth in comparable quantities [68]. The overall content of CBHs in the crude *P. verruculosum* preparation was approximately 70% of total proteins, while the content of endoglucanases and BGL was approximately 15 and 4%, respectively [68].

Based on the sequences of *Penicillium* GH7 CBHs and other characterized GH7 fungal enzymes of similar specificity retrieved from the CAZy database, a phylogenetic tree was constructed **(Figure 1)** [82,304]. The analyzed enzymes shared 44% of conserved amino acid positions. As can be seen from **Figure 1**, all *Penicillium* CBHs fell into enzyme groups located at the bottom part of the figure. They display highest similarity to CBHs from *Aspergilli* and slightly lower similarity to those from *Talaromyces emersonii* and *Thermoascus aurantiacus*. CBHs from other fungi located overhead, including the CBH I of *T. reesei*, share lower similarity with *Penicillium* enzymes. The highest similarity of the *Penicillium* CBHs to the enzymes from *Aspergillus*, *Talaromyces* and *Thermoascus* is not surprising, since all four genera listed belong to the same family of fungi (Trichocomaceae) [14].

Saccharification of lignocellulosic feedstock using *Penicillium* cellulases

Enzymatic conversion of cellulose to glucose is the most laborious step in the production of biofuels from

Table 2. Cellulases and β-glucosidases from *Penicillium* species belonging to different glycoside hydrolase families and whose complete or partial amino acid sequences are known.

GH family	Enzymes	Organism	Accession number†	Ref.
1	Three enzymes‡	*Penicillium chrysogenum*	B6GZU4, B6HAS4, B6H4K5	[60]
	Bgl1	*P. funiculosum*	ACO82080	
3	Bgl1	*P. brasilianum*	A5A4M8	[61]
	17 enzymes‡	*P. chrysogenum*	See CAZy database for numbers [303]	[60]
	Bgl1 (two versions)	*P. decumbens*	D3JUX2, B3GK87	[62]
	Bgl1§	*P. occitanis*	A7LKA2	
	Bgl§	*P. pinophilum*	NA	[69]
	Bgl3	*P. purpurogenum*	C9E9M9	[63]
5	EG	*P. brasilianum*	B8Q961	[46,64]
	Egl2	*P. canescens*	NA	[74]
	13 enzymes‡	*P. chrysogenum*	See CAZy database for numbers [303]	[60]
	Egl2 (two versions)	*P. decumbens*	A9Z054, AFG25592	[65]
	Egl1§	*P. echinulatum*	C5J4L7	[66]
	Egl2	*P. janthinellum*	Q12665	[67]
	Bgl2	*P. multicolor*	B5MEI8	
	Eng5	*P. pinophilum*	C0L2S4	
	EG§	*P. purpurogenum*	NA	[76]
	EG IIa and EG IIb§	*P. verruculosum*	NA	[68]
6	One enzyme‡	*P. chrysogenum*	B6H8F7	[60]
	CBH II	*P. decumbens*	ADX86895	
	Cellulase	*P. funiculosum*	B5TMG4	
	CBH II§	*P. verruculosum*	NA	[68]
7	Cbh1	*P. chrysogenum*	Q5S1P9	[25]
	Two enzymes‡	*P. chrysogenum*	B6HE71, B6HC69	[60]
	CBH I (two versions)	*P. decumbens*	C9EI49, A3RG86	
	Egl1 (two versions)	*P. decumbens*	B0FMT4, B6ZBT2	[65]
	Xylanase/CBH I (two versions)	*P. funiculosum*	Q8WZJ4, ADX60067	[70]
	CBH	*P. glabrum*	AEL78901	
	Cbh1	*P. janthinellum*	Q06886	[71]
	Cbh1	*P. occitanis*	Q68HC2	[72]
	Egl1	*P. oxalicum*	C5MRS3	
	Cbh1 (four versions)	*P. oxalicum*	See CAZy database for numbers [303]	
	EG	*P. purpurogenum*	AEL78899	
	CBH	*P. purpurogenum*	AEL78900	
	EG	*Penicillium* sp.	AEG74551	
	CBH I	*P. verruculosum*	NA	[68]
12	EGa§	*P. brasilianum*	NA	[64]
	Egl3	*P. canescens*	NA	[75]
	Three enzymes‡	*P. chrysogenum*	B6H819, B6H7Y5, B6HDE0	[60]
	Nonclassified§	*P. decumbens*	B5M080	
45	EG V	*P. decumbens*	B5AKD1	[73]
61	Four proteins‡	*P. chrysogenum*	See CAZy database for numbers [303]	[60]

†UniProtKB/TrEMBL or GenBank accession numbers are shown, if available.
‡Nonclassified enzymes; data from genome.
§Fragments of the amino acid sequence.
Bgl: β-glucosidase; CAZy: Carbohydrate-active enzyme; CBH/Cbh: Cellobiohydrolase; EG/Egl/Eng: Endoglucanase; GH: Glycoside hydrolase; NA: Data not available.
Data from [303].

lignocellulosic biomass. Cellulases from *Penicillium* species have successfully been applied for saccharification of different cellulose-containing materials [9,12,19,22,26,31,35,83–91]. The most interesting papers are those in which direct comparison of *Penicillium* and *Trichoderma* (*T. reesei*, as a rule) enzymes, equalized either by cellulase activity (FPA) or by protein concentration in the reaction system, have been carried out (Table 3).

In practically all cases *Penicillium* cellulases provided superior performance over commercial or laboratory *Trichoderma* enzyme preparations, when the latter were used without additional BGL. This was demonstrated

on various samples of pure cellulose, as well as on wood or agricultural lignocellulosic residues pretreated by different methods (Table 3). To the best of our knowledge, publications where the contrary situation would be observed (i.e., *Trichoderma* cellulases alone would provide higher cellulose conversions or glucose yields than cellulases from *Penicillium* species) are absent. One of the major reasons for the superior hydrolytic performance of extracellular multienzyme systems secreted by *Penicillium* species is their high BGL activity (other reasons are discussed in the subsequent section). Unlike in *T. reesei* where BGL are mostly intracellular

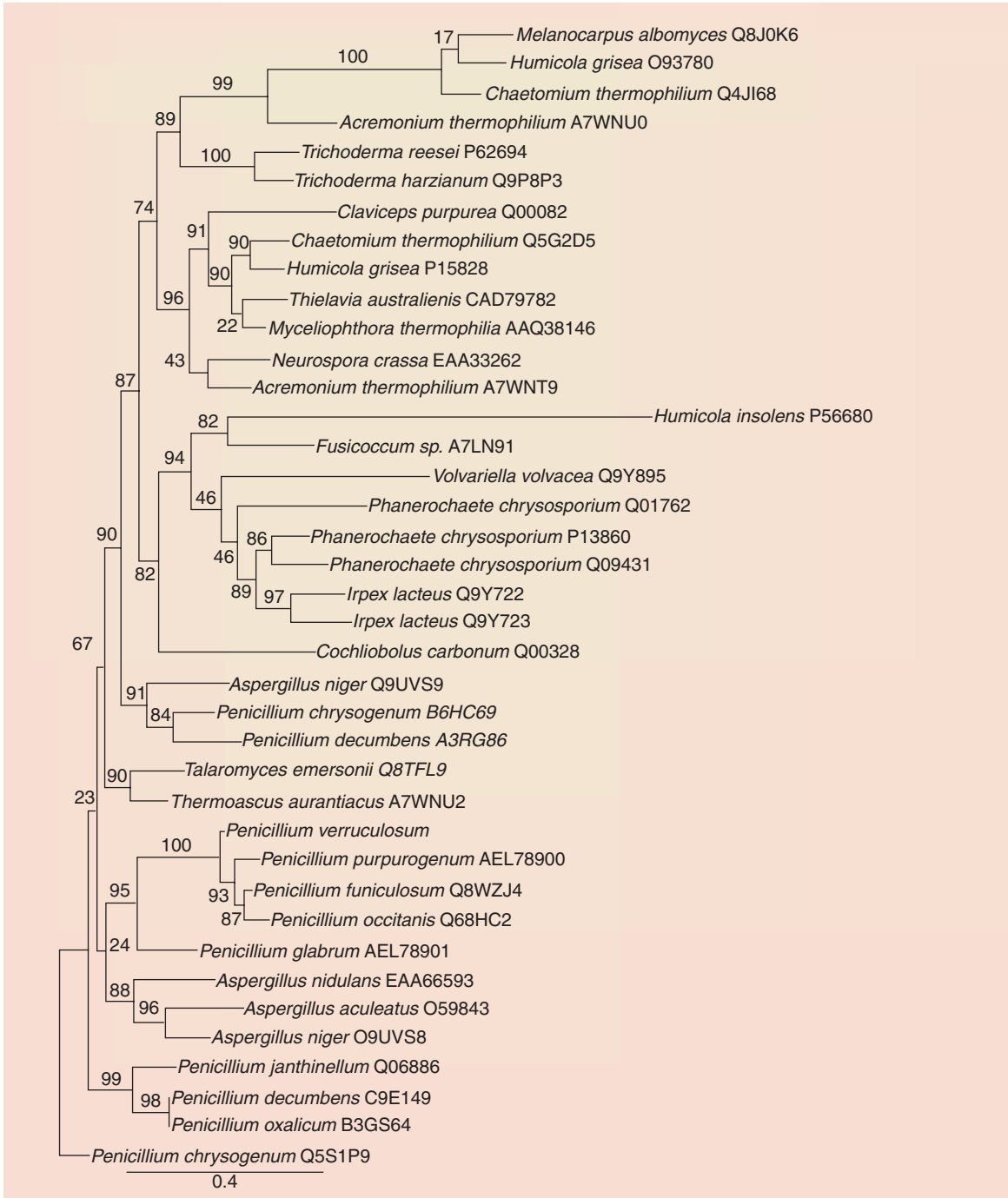

Figure 1. Phylogenetic tree for cellobiohydrolases from family 7 of glycoside hydrolases. Distances along the horizontal axis reflect the degree of relatedness of the sequences. Branch support values are shown in percentages. The phylogram was constructed using the Phylogeny.fr web service [82,304].

Table 3. Hydrolysis of cellulosic and lignocellulosic substrates using cellulases from *Penicillium* and *Trichoderma* species.

Feedstock	Conversion (%) or product concentration (g/l)		Hydrolysis conditions	Ref.
	Penicillium	*Trichoderma*		
Wood residues				
Steam-pretreated spruce; 38.5 g/l	44–48 or 55% (*Penicillium pinophilum*, *P. persicinum*, *P. brasilianum*)†	14 or 59% (Celluclast 1.5L)†	25 FPU/g cellulose; 40°C; pH 4.8; 24 h	[22]
	77–79% (*P. brasilianum*)	38 or 85% (Celluclast 1.5L)†	25 FPU/g cellulose; 40°C; pH 4.8; 69 h	[31]
Steam-exploded Douglas fir; 50 g/l	38 or 46% (*Penicillium* sp.)†	11–15 or 32–46% (six samples)†	10 FPU/g substrate; 50°C; pH 5.0; 12 h	[12]
Organosolv-pretreated Douglas fir; 50 g/l	25 or 40% (*Penicillium* sp.)†	10–16 or 27–41% (six samples)†		
Organosolv-pretreated lodgepole pine; 50 g/l	14 or 22% (*Penicillium* sp.)†	7–9 or 17–23% (six samples)†		
Organosolv-pretreated yellow poplar; 50 g/l	24 or 34% (*Penicillium* sp.)†	12–17 or 23–33% (six samples)†	10 FPU/g substrate; 50°C; pH 5.0; 12 h	[85]
Organosolv-pretreated red maple; 50 g/l	19 or 31% (*Penicillium* sp.)†	9–13 or 21–28% (six samples)†		
Organosolv-pretreated Douglas fir; 50 g/l	Glucose 25–31 g/l (*Penicillium verruculosum*, two samples)	11–12 or 19–23 g/l (Celloviridin, BioAce)†	10 mg protein/g substrate; 50°C; pH 5.0; 72 h	[9]
Unbleached eucalypt cellulose; 18 g/l	Reducing sugars 9.3–10.2 g/l (*P. verruculosum*, two samples)	2.6–6.9 g/l (five samples)	10 FPU/g substrate; 50°C; pH 5.0; 6 h	[86]
Organosolv-pretreated conifer wood; 50 g/l	Glucose 10.9–13.8 g/l (*P. verruculosum*, 4 samples); 8.7 g/l (*P. funiculosum* Rovabio® Excel)	2.7–8.4 g/l (eight samples)	10 FPU/g substrate; 50°C; pH 5.0; 12 h	[87]
Bleached eucalypt kraft pulp; 20 g/l	44% (*P. echinulatum*)	40 or 61% (Celluclast 1.5L)†	10 FPU/g substrate; 48 h	[88]
Agricultural residues				
Esparto grass cellulose	67% (*P. occitanis*)	37% (*Trichoderma reesei* QM9414)	50 FPU g/cellulose; 50°C; pH 4.8; 24 h	[19]
Milled corn cobs; 62.5 g/l	Glucose 23 g/l (*P. funiculosum*)	15–22 g/l (GC 200, Spezyme CP, Celluclast 1.5L)	11 FPU/g substrate; 45°C; pH 4.8; 48 h	[35]
Industrial wastes or pure cellulose				
Short fiber cellulose wastes; 100 g/l	Glucose 50 g/l (*Penicillium* sp.)	37 or 45 g/l (Celloviridin)†	2 FPU/ml; 50°C; pH 4.5; 24 h	[83]
Cellolignin; 150 g/l	Glucose 49 g/l (*Penicillium* sp.)	23 or 40 g/l (Celloviridin)†		
Microcrystalline cellulose; 100 g/l	Glucose plus cellobiose 45 g/l (*Penicillium* sp.)	25 or 36 g/l (Celloviridin)† 34 or 41 g/l (Cytolase 300)†	1 FPU/ml; 50°C; pH 4.5; 24 h	[84]
Avicel; 50 g/l	Glucose 36–38 g/l (*P. verruculosum*, two samples)	23–27 or 41–42 g/l (Celloviridin, BioAce)†	10 mg protein/g substrate; 50°C; pH 5.0; 72 h	[9]
α-cellulose from bagasse; 100 g/l	67–100% (*P. pinophilum*)	20–77% (Accellerase 1000)	5–20 FPU/g substrate; 50°C; pH 4.8; 48 h	[89]
Solka-Floc cellulose; 100 g/l	28–55% (*P. pinophilum*)	25–48% (Accellerase 1000)		
CP-123 cellulose; 100 g/l	21–58% (*P. pinophilum*)	16–30% (Accellerase 1000)		
Sigmacell cellulose; 100 g/l	26–46% (*P. pinophilum*)	9–26% (Accellerase 1000)		

†The last numbers show conversion or product concentration after the addition of surplus β-glucosidase.
FPU: Filter paper unit.

[6,8,39], *Penicillia* secrete relatively high levels of the BGL activity into a culture liquid (Table 1).

In many cases listed in Table 3, a BGL from an external source (typically from *Aspergillus* sp.) was added to *Trichoderma* cellulases to improve their performance. After the addition of the surplus BGL, the *Trichoderma* preparations sometimes provided higher cellulose conversions or glucose yields than *Penicillium* cellulases.

Such a situation was reported for steam pretreated spruce [22,31], bleached eucalypt kraft pulp [88] and Avicel [9]. In a few other cases, *Penicillium* enzymes retained preference or displayed similar performance, compared with the *Trichoderma* cellulases enhanced with the BGL [9,12,83–85]. The superior performance of *P. pinophilum* cellulases over the modern Accellerase 1000 preparation (from Genencor), produced by a genetically modified *T. reesei* strain and possessing a high BGL activity, has been reported [89]. These data deserve special attention, since different cellulosic substrates were hydrolyzed at different enzyme dosages (5–20 FPU/g substrate) for various time (16–96 h, only data for 48 h are shown; **Table 3**), and for all enzyme loadings and reaction times *P. pinophilum* cellulases displayed a notable preference over Accellerase 1000 [89].

Therefore, it is not surprising that in some studies either crude enzyme preparations or individual BGLs from *Penicillia* have been used for supplementation of BGL-deficient *Trichoderma* cellulases in order to improve the saccharification performance of the latter [62,90–93]. Van Wyk observed a pronounced synergistic effect between enzyme preparations from *T. viride* and *P. funiculosum* in hydrolysis of microcrystalline cellulose, filter paper, foolscap paper and newsprint [90]. Crude enzyme systems secreted by six different fungi, including *P. funiculosum*, were used for boosting enzymatic degradation of barley straw by *T. reesei* preparation (Celluclast) at different temperatures (35–60°C) [92]. One of the most pronounced boosting effects was observed in the case of *P. funiculosum* enzymes at 50°C, the temperature optimum for *T. reesei* cellulases. Using different blends of commercial Multifect® CX 10L preparation (from Genencor) with enzyme extracts produced by *P. funiculosum* and *Trichoderma harzianum*, enzymatic hydrolysis of pretreated sugarcane bagasse was studied [91]. The most efficient enzyme cocktail was prepared by mixing the Multifect preparation with *P. funiculosum* enzymes (1:1 by FPA); it provided hydrolysis yields above 97%, while the *P. funiculosum* extract alone was the best among individual enzyme preparations. Purified BGLs from *P. decumbens* [62] and *P. citrinum* [93] were used for improving hydrolysis of corncob residues and filter paper by *T. reesei* cellulases. Up to 3.4-fold improvements in glucose yields were achieved.

Other routes for obtaining multienzyme systems with high cellulose saccharification ability include genetic transformation or protoplast fusion between fungal species. The *P. decumbens* BGL was introduced into the genome of *T. reesei* Rut C30; as a result, the BGL activity of enzyme complexes secreted by two selected transformants increased six–eight-fold, while the FPA was enhanced by 30% in comparison to the parental *T. reesei* strain [94]. Dillon *et al.* [95] carried out protoplast fusion between *P. echinulatum* and *T. harzianum*. Some of the resulting fusants, morphologically more similar to the parental *P. echinulatum*, presented higher FPA and BGL activities compared with the parental strains.

Reasons for high hydrolytic efficiency of multienzyme systems secreted by *Penicillia*

The high BGL activity of enzyme complexes secreted by *Penicillium* species was notified by practically all researchers studying cellulases from these fungi. The contribution of this factor into cellulose saccharification ability of *Penicillia* was already discussed in the preceding sections. However, other reasons for the superior performance of *Penicillium* cellulases in hydrolysis of lignocellulosic materials exist, which are less evident and not widely covered by numerous publications. One of them seems to be a higher specific activity of key enzymes in cellulose biodegradation and, in particular, that of CBHs [9,68,201]. Other properties of cellulases, such as thermostability, susceptibility to product inhibition, adsorption on lignin or inhibition by lignin-derived compounds, may also be important [46,72,76,96–102].

In the hydrolysis of pretreated corn stover (4.3%), purified CBH I from *P. funiculosum* (27.8 mg/g cellulose), used in combination with *Acidothermus cellulolyticus* EG (1.13 mg/g cellulose), provided 69% conversion of cellulose after 72 h of the reaction, versus 52% conversion for the native CBH I from *T. reesei* with the *A. cellulolyticus* EG at the same protein loadings [201]. An equivalent loading of the recombinant *P. funiculosum* CBH I expressed in *Aspergillus awamori* yielded 65% conversion of cellulose in 72-h hydrolysis. Even at one-half loading (13.9 mg/g cellulose) the performance of the recombinant CBH I (62% conversion) was greater than that of the *T. reesei* CBH I at full loading.

Extremely high hydrolytic performance has also been reported for the intact high-molecular weight forms of CBH I and CBH II from *P. verruculosum* in hydrolysis of Avicel in the presence of purified *Aspergillus japonicus* BGL [68]. Both enzymes were notably more effective than the respective CBHs I and II of *T. reesei* **(Figure 2)**. It is noteworthy that the performance of the catalytic module of the *P. verruculosum* CBH II (its low-molecular-weight form without a CBM) was only slightly worse than that of its intact form but better than the performance of the native *T. reesei* CBH II. The catalytic modules of cellulases usually display significantly lower activities against crystalline cellulose in comparison with full-size enzymes possessing a CBM [9,68]. The intact CBH I from *P. verruculosum* demonstrated even

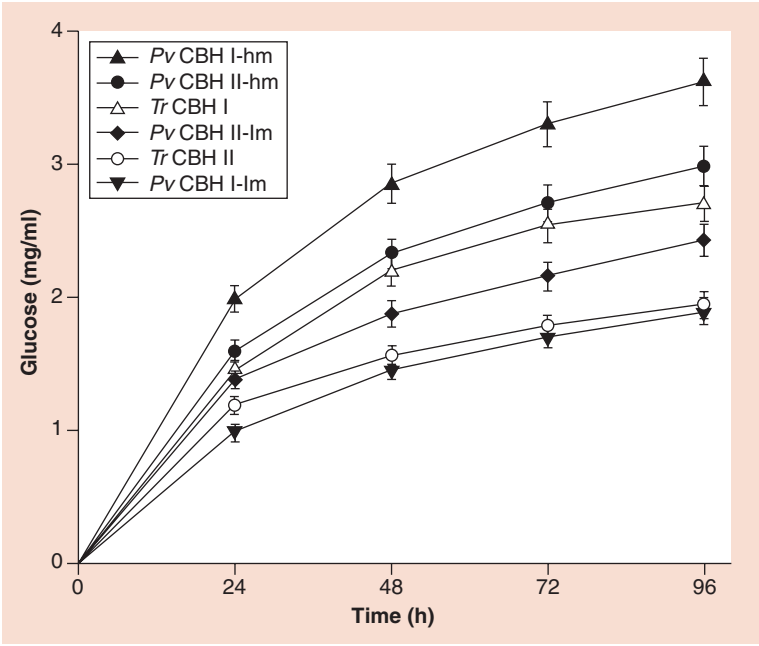

Figure 2. Progress kinetics of Avicel (5 mg/ml) hydrolysis by purified cellobiohydrolases from *Penicillium verruculosum* and *Trichoderma reesei*.
In the presence of purified *Aspergillus japonicus* β-glucosidase (0.5 U/ml) at 40°C, pH 5.0, protein loading of 0.1 mg/ml.
hm: High-molecular-weight enzyme form; lm: Low-molecular-weight enzyme form; Pv: *Penicillium verruculosum;* Tr: *Trichoderma reesei*.
Data from [9,68].

higher benefits over the *T. reesei* CBHs in hydrolysis of lignocellulosic feedstocks, such as pretreated corn residues and sugarcane bagasse [Gusakov AV, Sinitsyn AP, Unpublished Data]. It is interesting to note that the enzymes from *P. funiculosum* and *P. verruculosum*, whose extremely high saccharification performance has been documented, share a high degree of identity (**Figure 1**; lines 10 and 12 from the bottom).

Examples of the EG from *P. purpurogenum* [76] and BGL from *P. pinophilum* [69], possessing extraordinary specific activities against CMC and *p*-nitrophenyl-β-D-glucopyranoside, have also been reported. The first enzyme displayed notably higher thermostability (half-life time of 2 h at 70°C) in comparison with other typical cellulases from mesophilic fungi.

One of the factors negatively affecting the enzymatic conversion of cellulose to sugars is enzyme inhibition by the reaction products. The most pronounced is the inhibition of CBHs by cellobiose. The inhibition constants for different fungal GH7 family CBHs have typically been estimated using synthetic chromogenic derivatives of cellobiose or lactose as a substrate. The CBH I from *P. occitanis* was found to be less sensitive to inhibition by cellobiose (K_i = 2 mM) than most of other characterized enzymes of similar specificity [97]. For a comparison, the reported value of the competitive inhibition constant for *T. reesei* CBH I is 0.02 mM, while for five CBHs from *Phanerochaete chrysosporium*, *Myceliophthora thermophila* (formerly classified as *Chrysosporium lucknowense*), *T. emersonii* and *Humicola insolens* the K_i value varied in the range of 0.07–0.65 mM [98,99]. CBHs with reduced inhibition sensitivity to cellobiose, similar to the *P. occitanis* CBH I, have also been described; they include CBH I, CBH II from *Coniophora puteana* and CBH IA from *T. emersonii* with K_i values of 1.2, 2.4 and 2.5 mM, respectively [98]. Structural modeling showed that the reason for the relative insensitivity of *P. occitanis* CBH I to product inhibition was poor hydrogen bonding and a more open configuration of the active site [72].

Data on inhibition of the GH6 family CBHs by cellobiose are scarce since these enzymes do not possess an activity against chromogenic derivatives of disaccharides; this makes the precise registration of the reaction product in the presence of added cellobiose very difficult. However, Gao *et al.* [100] described the CBH II from *P. decumbens* that displays an activity against *p*-nitrophenyl-β-cellobioside and seems to be the first GH6 family CBH possessing a GH7 CBH I-like type of activity. The *P. decumbens* CBH II was competitively inhibited by cellobiose with a K_i value of 44 mM; that is, it was less sensitive to the product inhibition than the characterized fungal CBHs from the GH7 family.

Another crucial factor negatively affecting the performance of cellulases in lignocellulose hydrolysis is nonproductive binding of the enzymes to lignin and, thus, their inhibition by this biomass component [101,102]. In hydrolysis of Avicel, cellulases from *Penicillium* sp. were notably less inhibited by two types of lignin, artificially added to the reaction system at the ratio Avicel:lignin of 1:1, than five *T. reesei* cellulase preparations from different sources; the organosolv dissolved lignin induced greater reduction in hydrolysis rate (11–84%) than the residual lignin obtained by the exhaustive enzymatic hydrolysis of pretreated wood (8–58% rate reduction) [102]. Similar lower susceptibility of *Penicillium* sp. enzymes to the inhibition by softwood and hardwood-derived lignins in hydrolysis of α-cellulose, in comparison to *T. reesei* cellulase (commercial Celluclast 1.5L preparation), was reported [101].

Three cellulase samples, obtained by cultivation of *P. brasilianum* on different carbon sources and demonstrating twofold higher glucose production in 69-h hydrolysis of steam pretreated spruce than Celluclast 1.5L, displayed 70–80% adsorption on this lignin-rich substrate, while the *T. reesei* enzymes from Celluclast 1.5L bound to the substrate almost completely (by

94–98%) [31]. Since the degree of adsorption of *P. brasilianum* enzymes on pure Sigmacell cellulose was almost threefold lower, the researchers concluded that the enzyme binding to lignin component of the substrate, due to hydrophobic interactions, takes place in the case of pretreated spruce. The capillary electrophoresis analysis showed that *P. brasilianum* GH12 EGa without a CBM binds to the lignin-containing substrate to a lesser extent compared with other proteins. This result is in good agreement with data of Berlin *et al.* [101,102], who also observed lower binding of *Penicillium* GH12 EG III to lignins and lower susceptibility of the enzyme to inhibition by lignin than other cellulases (in particular, those from *T. reesei*).

Krogh *et al.* [46] studied the adsorption of the purified GH5 EG from *P. brasilianum* on nine samples of lignin; most of them were obtained by extensive enzymatic digestion of wood and agricultural residues using commercial cellulase preparations. The enzyme was incubated with residual lignins (25 g/l) for 3 h at 4°C and pH 4.8 using protein loading of 10 mg/g substrate. The adsorption was in the range of 5–33% for most lignin samples, while on lignin derived from acid-free steam-exploded rice straw it was dramatically higher (82%). The fact that the degree of enzyme adsorption on lignin (and, hence, the extent of inhibition of the enzyme activity by lignin) depends on its source and method of preparation was explained by differences in physical properties and chemical compositions of different lignin samples. In particular, the organosolv dissolved lignin had lower content of carboxyl and hydroxyl groups than that obtained by the exhaustive hydrolysis of pretreated wood, suggesting higher surface hydrophobicity [102]. Much higher adsorption of the *P. brasilianum* EG on residual lignin obtained from rice straw could be explained by relatively high ash (silica) content in the straw (up to 15%) [46].

Other reasons for high saccharification performance of *Penicillium* cellulase systems may exist, which are less evident, but should still be found. So far, practically nothing is known about GH61 family proteins, which have been found in *P. chrysogenum* genome **(Table 2)** and may likely be present in other *Penicillium* species. These proteins are known to act synergistically with cellulases [80,81]. Very recent publications demonstrated that the GH61 proteins from *T. aurantiacus* [78] and *P. chrysosporium* [79] are copper-dependent oxidoreductases that cleave cellulose through the oxidative mechanism.

Future perspective

Extracellular cellulase systems secreted by various *Penicillium* species proved to be efficient destroyers of cellulose and, thus, they are very promising candidates for use in the processes of bioconversion of lignocellulosic feedstocks to liquid fuels. However, in order to accomplish the cost-competitive large-scale production of second-generation biofuels, some tasks should be solved. One of the most important issues is the enzyme production cost. Big efforts have been made in the last decade to reduce the cost of cellulases and, in particular, those produced by *T. reesei* genetically modified strains. A recent estimation of the *T. reesei* cellulase cost for bioethanol production is approximately US$0.5 per gallon of cellulosic ethanol [13]; data on the production cost of the *Penicillium* enzymes are not available so far. Although some *Penicillium* strains are able to secrete protein levels comparable to *T. reesei* high cellulase producers in laboratory-scale fermenters (in the order of ~40–50 g/l), the modern *T. reesei* strains still seem to be better; able to produce approximately 100 g/l of extracellular protein in industrial fermentations [39]. Further optimization and reduction of the cost of the fermentation medium, elucidation of mechanisms of enzyme induction and regulation in *Penicillia*, as well as obtaining genetically modified strains with increased biosynthesis of cellulases and accessory enzymes, may be needed in order to make the production of biofuels from lignocellulosic biomass, based on *Penicillium* enzymes, economically feasible.

Some of *Penicillium* cellulases possess unique properties in comparison with other known microbial enzymes, such as a higher specific activity, lower sensitivity to the product inhibition and weaker adsorption on lignin component of the biomass (reduced inhibition by lignin). These enzymes may be potential candidates for heterologous expression in high-productive hosts, together with other cellulases and hemicellulases hydrolyzing the biomass polysaccharides or acting as accessory enzymes to them. Alternatively, *Penicillium* strains with extraordinary cellulose saccharification ability, high levels of BGL and overall protein production may become hosts for heterologous expression of useful foreign enzymes/proteins to further increase the hydrolytic potential of these fungi.

Currently, relatively little information is known about the full pattern of cellulolytic and hemicellulolytic genes in *Penicillia*. Although the genome of *P. chrysogenum* Wisconsin 54–1255 strain has recently been sequenced, properties of the enzymes found in the genome remain unstudied; most of them are not even classified from the point of view of their substrate specificity. Sequencing genomes of other *Penicillium* species, their annotation, purification and characterization of encoded enzymes will likely allow finding novel enzymes with interesting properties useful for biofuel production.

Financial & competing interests disclosure

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Executive summary

Cellulase production by *Penicillium* species

- Various *Penicillium* species secrete extracellular multienzyme systems that efficiently destroy the cellulose component of plant biomass.
- Cellulase production up to 13 filter paper units per ml, β-glucosidase production up to 61 U/ml and protein production up to 47 g/l in laboratory-scale fermenters have been reported for *Penicillium* mutant strains.

Enzyme induction & regulation

- Mechanisms of induction and regulation of cellulase biosynthesis have not been studied very thoroughly in *Penicillia* as they have been for other fungi. However, it seems that these mechanisms are rather universal. The induction and expression of hemicellulolytic and cellulolytic enzyme systems may be mediated by the XlnR transcriptional activator, while the CreA transcriptional regulator is responsible for the carbon catabolite repression.

Cellulolytic system of *Penicillium* fungi

- The cellulase system of *Penicillium* species is rather typical for fungi. It consists of a few endoglucanases belonging to the glycoside hydrolase (GH) families 5, 6, 7, 12 and 45, two or more GH6 and GH7 cellobiohydrolases, and GH1 and GH3 β-glucosidases.
- The GH7 family cellobiohydrolases from *Penicillia* display highest similarity to cellobiohydrolases from *Aspergilli*, and slightly lower similarity to those from *Talaromyces emersonii* and *Thermoascus aurantiacus*.

Saccharification of lignocellulosic feedstock using *Penicillium* cellulases

- When equalized either by cellulase activity or by protein concentration in the reaction system, enzyme preparations from *Penicillia* usually display higher saccharification performance in hydrolysis of different lignocellulosic feedstocks than cellulase preparations produced by modern mutant strains of *Trichoderma reesei*.

Reasons for high hydrolytic efficiency of multienzyme systems secreted by *Penicillia*

- One of the major reasons for higher saccharification performance of *Penicillium* cellulases is a high level of the β-glucosidase activity. Other reasons include extremely high specific activities of key enzymes responsible for cellulose biodegradation (in particular, cellobiohydrolase activity), lower sensitivity of cellobiohydrolases to product inhibition, as well as weaker nonproductive binding of enzymes to the lignin component of the biomass.

Future perspective

- Cellulases from *Penicillium* species are very promising candidates for use in the processes of bioconversion of lignocellulosic feedstocks to liquid fuels, although higher protein biosynthesis levels as well as lower enzyme production costs should probably be achieved in order to make these enzymes more economically competitive.

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