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MINI-REVIEW

Regulation of genes encoding polysaccharide-degrading enzymes in *Penicillium*

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Abstract

Penicillium fungi, including *Penicillium oxalicum*, can secrete a range of efficient plant-polysaccharide-degrading enzymes (PPDEs) that is very useful for sustainable bioproduction, using renewable plant biomass as feedstock. However, the low efficiency and high cost of PPDE production seriously hamper the industrialization of processes based on PPDEs. In *Penicillium*, the expression of PPDE genes is strictly regulated by a complex regulatory system and molecular breeding to modify this system is a promising way to improve fungal PPDE yields. In this mini-review, we present an update on recent research progress concerning PPDE distribution and function, the regulatory mechanism of PPDE biosynthesis, and molecular breeding to produce PPDE-hyperproducing *Penicillium* strains. This review will facilitate future development of fungal PPDE production through metabolic engineering and synthetic biology, thereby promoting PPDE industrial biorefinery applications.

Key points

- *This mini review summarizes PPDE distribution and function in Penicillium.*
- *It updates progress on the regulatory mechanism of PPDE biosynthesis in Penicillium.*
- *It updates progress on breeding of PPDE-hyperproducing Penicillium strains.*

Keywords *Penicillium oxalicum* · Plant-polysaccharide-degrading enzyme · Regulatory factor · Molecular breeding

Introduction

Plant polysaccharides are the most abundant renewable resource on earth, with cellulose, hemicellulose, and starch being the major types. All three can be converted into high-value-added sustainable bioproducts by industrial biorefineries. During biorefining, plant-polysaccharide-degrading enzymes (PPDEs), as sustainable biocatalysts, are indispensable and environmentally friendly (Hemati et al. 2022; Li et al. 2022a; Ning et al. 2021). PPDEs are a class of enzymes that can hydrolyze plant polysaccharides into monosaccharides or oligosaccharides under mild conditions; cellulase, xylanase, and amylase account for the majority of the industrial enzyme market (Dharma Patria et al. 2022).

Cellulase and xylanase are widely used for degradation of cellulose and xylan, which are the main structural materials in plant cell walls, to release mono- or oligosaccharides, such as glucose, xylooligosaccharides, and xylose. Cellulases are classified into three types, based on their modes of action, i.e., endo-β-1,4-glucanase (EG, EC 3.2.1.4), cellobiohydrolase (CBH, EC 3.2.1.91), and β-glucosidase (BGL, EC 3.2.1.21). EG randomly breaks internal β-1,4-glycosidic bonds in amorphous regions of cellulose chains, providing more chain termini that can then be hydrolyzed by CBH (Fig. 1). CBH is an exo-acting enzyme that hydrolyzes cellulose chains at the ends, releasing cellobiose. BGL, another exo-acting enzyme, hydrolyzes cellobiose and cello-oligosaccharides into glucose (Sukumaran et al. 2021; Wang et al. 2020a).

There are two classes of xylanase, endo-β-1,4-xylanase (XYN, EC 3.2.1.8) and β-xylosidase (XYL, EC 3.2.1.37).

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XYN hydrolyzes β-1,4-glycosyl bonds in xylan, generating xylo-oligosaccharides, whereas β-xylosidase hydrolyzes the nonreducing ends of xylo-oligosaccharides into xylose (Fig. 1) (Wang et al. 2020a; Mendonça et al. 2023).

Raw-starch-degrading enzyme (RSDE) is capable of degrading raw starch granules into glucose, below the gelatinization temperature of starch. RSDEs include raw-starch-degrading α-amylase (RSDA; EC 3.2.1.1), raw-starch-degrading β-amylase (EC 3.2.1.2), and raw-starch-degrading glucoamylase (RSDG; EC 3.2.1.3) (Sun et al. 2008, 2010; Fang et al. 2019a; Xu et al. 2016). RSDEs generally contain starch-binding domains. RSDA randomly cleaves internal α-1,4-glycosidic linkages in starch granules whereas exo-acting RSDG breaks α-1,4- and α-1,6-glycosidic bonds to release glucose (Fig. 1) (Zhao et al. 2022a).

In addition, lytic polysaccharide monooxygenases (LPMOs), a class of copper ion-dependent oxidases, have the auxiliary function of degrading polysaccharides that are resistant to hydrolytic enzymes, by oxidative cleavage of glycosidic bonds. Indeed, the LPMOs, auxiliary activity family (AA) 9, AA14, and AA13 have catalytic activity against cellulose, xylan, and starch, respectively (Fig. 1) (Guo et al. 2022).

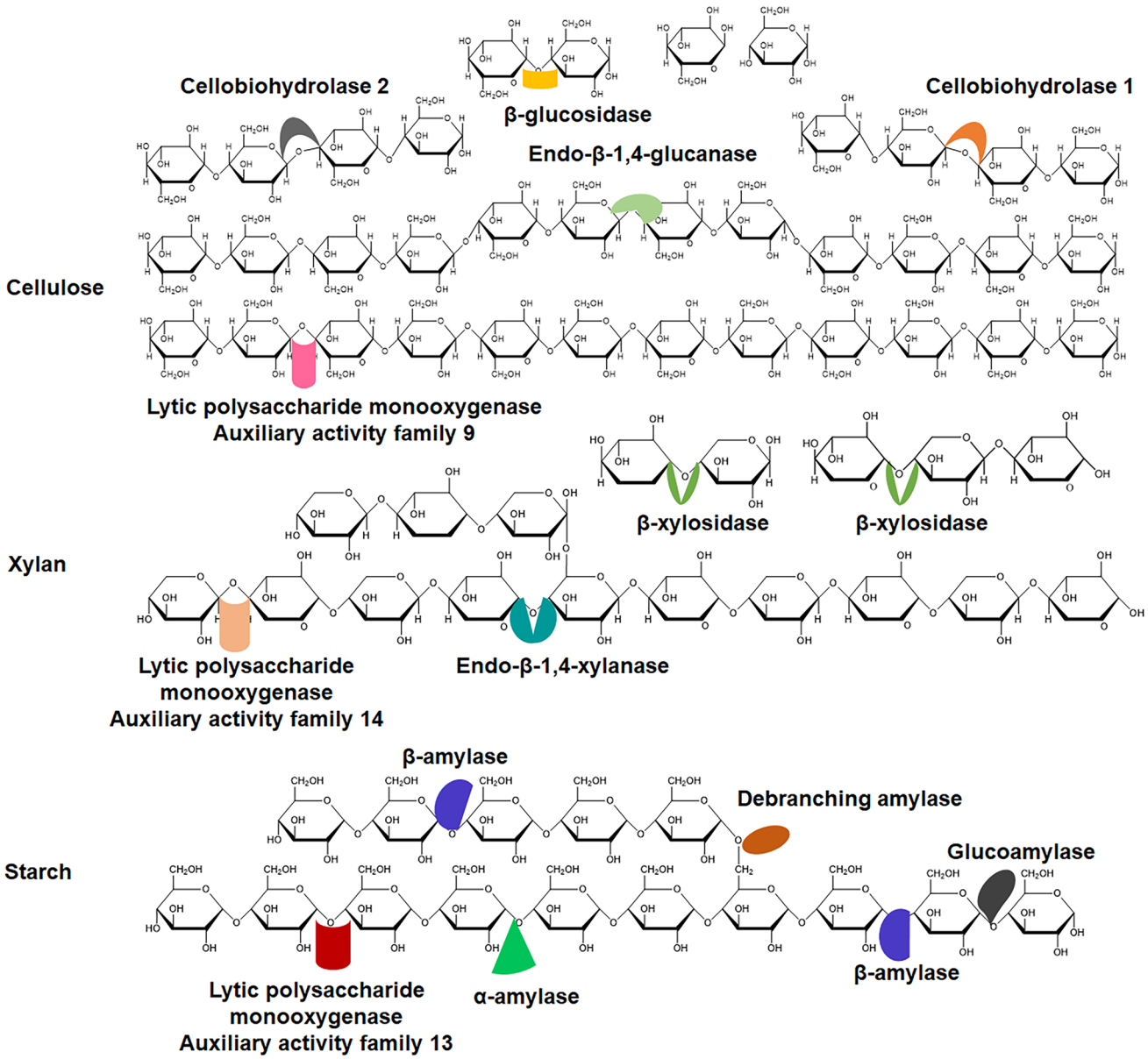

Fig. 1 Modes of action of plant polysaccharide-degrading enzymes from *Penicillium* on cellulose, xylan, and starch. Scheme shows the bond-cleavage specificity of each enzyme

Notably, efficient hydrolysis of plant polysaccharides requires the synergistic action of various PPDEs in a suitable ratio, which is a complex process, depending on the types and proportions of the different substrates and enzymes, and their interactions (Monclaro et al. 2022; Wang et al. 2020a).

To date, many microorganisms that can secrete PPDEs have been reported, including bacteria, archaea, and filamentous fungi (Behera et al. 2017). Filamentous fungi, such as *Penicillium* and *Trichoderma*, have evolved relatively efficient protein expression and secretion systems, and are preferable for PPDE production (Wang et al. 2020b; Passos et al. 2018; Liu et al. 2012). The superior cellulase producer, *Trichoderma reesei* RUT-C30, secretes a relatively large amount of protein. Nevertheless, the cellulase mixture produced by RUT-C30 degrades cellulose inefficiently, as a result of insufficient β-glucosidase activity (Fang et al. 2019b). Unlike *T. reesei*, *Penicillium* strains, specifically *Penicillium oxalicum*, secrete an extraordinary cellulase mixture with a high β-glucosidase activity, which is recognized as superior to that of *T. reesei* (Li et al. 2017; Wang et al. 2021; Vaishnav et al. 2018). In addition, accumulated evidence indicates that *P. oxalicum* also produces high RSDE activity during induction by starch (Zhao et al. 2022b; Gu et al. 2020). Notably, the PPDEs produced by *P. oxalicum* have high degradation efficiency against plant polysaccharides, including cellulose from sugarcane bagasse and raw starch from cassava and corn (Zhao et al. 2021; Zhao et al. 2023a; Gu et al. 2020; Zhao et al. 2022a).

In this review, we summarize and analyze recent research advances on PPDE production in *Penicillium* (specifically *P. oxalicum*), regulation of PPDE biosynthesis, and molecular breeding for enhancing PPDE production.

PPDEs in *P. oxalicum* and other *Penicillium* species

Penicillium is a very large and ecologically diverse fungal genus, including more than 483 species (Petersen et al. 2023). Some of them can secrete complete and highly active PPDEs. However, because of a lack of genetic background information, understanding and exploration of these species and their enzyme systems still face many challenges. In the GenBank database (https://www.ncbi.nlm.nih.gov/genome), approximately 25% of the entries have been genome-sequenced, including *P. oxalicum* (Zhao et al. 2016; Li et al. 2022b; Liu et al. 2013a; Pham et al. 2023), *Penicillium echinulatum* (Lenz et al. 2022a), *Penicillium ucsense* (Lenz et al. 2022b), *Penicillium expansum* (Wu et al. 2019), *Penicillium solitum* (Wu et al. 2019), and *Pencillium parvum* (Long et al. 2023).

Annotation of protein functions indicates that *Penicillium* genomes contain many carbohydrate-active enzymes (CAZymes), but the number of enzymes varies between strains. For example, *P. oxalicum* strain HP7-1 annotates 712 CAZymes, including 271 glycoside hydrolases (GHs), 178 glycosyl transferases (GTs), 131 AAs, 80 carbohydrate-binding modules (CBMs), 106 carbohydrate esterases (CEs), and 25 polysaccharide lyases (Li et al. 2022b). In comparison, the *P. oxalicum* strain I1R1 genome annotates 653 CAZymes, including 312 GHs, 170 GTs, 72 AAs, 44 CBMs, 44 CEs, and 11 PLs (Pham et al. 2023). *P. parvum* strain 4-14 annotates 404 CAZymes, including 221 GHs, 94 GTs, 44 AA, 28 CBMs, 14 CEs, and three PLs (Long et al. 2023). Notably, statistical analysis indicates no significant difference in the frequency of CAZymes among the species in genus *Penicillium*, whereas the frequency of CAZymes varies greatly among species in phylum Ascomycota. Overall, the predicted number of CAZymes in *Penicillium* is higher than in *Trichoderma*, suggesting that *Penicillium* is a better source of enzymes for plant biomass saccharification (Lenz et al. 2022a). Nevertheless, the number of CAZymes in a fungal species does not necessarily reflect the efficiency of plant biomass breakdown; efficiency depends on the composition of the enzyme mixture and the relative proportion of each enzyme.

Some reports on genome annotation concern the major PPDEs, specifically cellulase, xylanase, and amylase, and their applications. Most reports focus on *P. oxalicum*, for example, *P. oxalicum* strain HP7-1 has genes for 25 cellulases (three CBHs, 10 EGs and 12 BGLs), 10 XYNs, and 12 amylolytic genes (three α-amylases, three glucoamylases, five α-glucosidases, and one 1,4-α-glucan branching enzyme) (Li et al. 2022b). *P. parvum* strain 4-14 has genes for 24 cellulases (three CBHs, six EGs and 15 BGLs), six XYNs, and 18 amylolytic genes (three α-amylases, five glucoamylases, and 10 α-glucosidases) (Long et al. 2023). Remarkably, the major PPDEs (cellulases CBH1, EG1, EG2, BGL1; XYNs Xyn11A, and Xyn10A and amylases Amy15A and Amy13A) are relatively highly conserved and are present in all the annotated *Penicillium* genomes.

To sum up, *Penicillium* has more abundant PPDE genes, its enzyme system is more diversified, and it can adapt to more abundant induced carbon sources and produce cellulase when induced by a variety of different carbon sources.

Diverse regulatory mechanisms of PPDE gene expression in *P. oxalicum* and other *Penicillium* species

The expression of PPDE genes in *Penicillium* is known to be strictly regulated by a complex regulatory network. PPDE biosynthesis depends on induction by nonpreferred carbon sources, for example, Avicel for cellulase, xylan for xylanase, and starch for RSDE. Regulation of PPDE biosynthesis

is very complex, with multiple levels and influencing factors, including carbon source, culture conditions, as well as transcriptional and (post-)translational regulation. Most reports refer to regulation by transcription factors (TFs) in *Penicillium*, specifically *P. oxalicum*.

Different types of PPDE, including cellulase, xylanase, and RSDE, are regulated by co-shared, or specific TFs, with diverse regulatory mechanisms at the transcriptional level. These TFs and their targets form a web-like network that dynamically modulates gene expression and respond to induction by different polysaccharide substrates. Numerous TFs that regulate PPDE expression in *P. oxalicum* have been identified (Fig. 2). Overall, TFs are classified into two types, repressor and activator. For example, two master transcriptional repressors, CreA (Li et al. 2015) and CxrC (Zhang et al. 2021), function through both direct and indirect mechanisms. Both can inhibit the expression of major PPDE genes, including cellulase genes (e.g., *cbh1*, *eg1*, *eg2*, and *bgl1*), xylanase genes (e.g., *xyn11A* and *xyn10A*), and amylase genes (*PoxGA15A*/*amy15A* and *amy13A*), as well as their regulatory genes such as *clrB*, *xlnR*, and *amyR*, in the presence of specific carbon sources (Li et al. 2015; Zhao et al. 2022c). In addition, CreA mediates carbon catabolite repression (CCR), whereas CxrC is not involved in CCR.

CreA contains a C2H2-type zinc finger domain and recognizes a conserved DNA motif (5′-SYGGRG-3′) (Cupertino et al. 2015), whereas the recently developed MEME-ChIP assay (https://meme-suite.org/meme/doc/meme-chip.html?man_type=web) found that CreA binds to DNA with a core sequence of CGGG in *A. nidulans*. However, further research will be needed to elucidate whether DNA binding involves direct action by CreA, or indirect interaction of CreA with other regulators (Chen et al. 2021). CreA is known to recruit the co-repressor complex, Tup1-Cyc8, which interacts with the major RNA Pol II subunit, thereby preventing transcription initiation. A histone methyltransferase, Set2, methylates H3K36, which involves CreA repression via interaction with Cyc8 in *P. oxalicum* (Hu et al. 2021a). In addition, the action of CreA is also controlled by post-translational modifications, such as phosphorylation (Alam et al. 2017; Ribeiro et al. 2019; Chen et al. 2021).

CxrC contains a Zn(II)2Cys6 binuclear cluster DNA-binding domain, which recognizes a core DNA sequence, TSSGTYR (S: C and G; Y: T and C; R: G and A), determined by in vitro electrophoretic mobility shift assay (EMSA) combined with a MEME assay. Homodimerization of CxrC was demonstrated in vitro, requiring both N- and C-termini and phosphorylation. Furthermore, a conserved oligopeptide LPSVRSLLTP (65–74) is essential for CxrC action (Zhang et al. 2021).

Recently, a novel transcriptional repressor, CxrD was found to downregulate cellulase and xylanase production in *P. oxalicum*, when solid-state fermented on a medium containing wheat bran and rice straw as carbon sources. CxrD does not participate in CCR, but dynamically regulates the expression of major cellulase and xylanase genes, such as *cbh1*, *eg1*, *bgl1*, and *xyn11A*, by binding to a core DNA sequence, 5′-CYGTSW-3′ (Zhao et al. 2023b).

In addition to transcriptional repressors, many transcriptional activators have been identified, which correspond to different types of PPDEs. For example, ClrB, which contains a Zn2Cys6-type DNA binding domain, is considered to be the master activator of cellulolytic gene expression, but is not required for xylanase gene expression in *P. oxalicum*

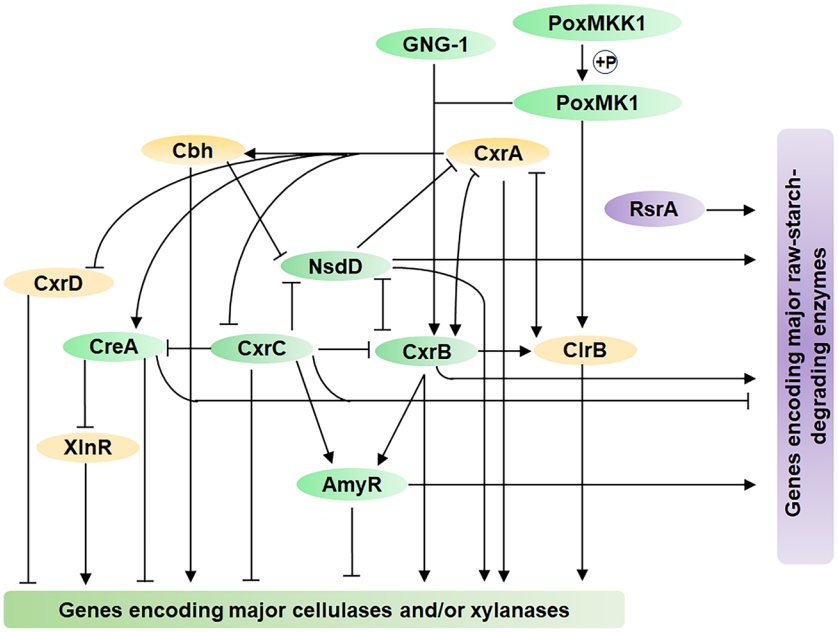

Fig. 2 Gene expression regulatory network of genes encoding major plant polysaccharide-degrading enzymes in *Penicillium oxalicum*. Regulatory proteins shaded orange, purple, and aqua green indicate involvement in regulation of cellulase and/or xylanase genes, raw-starch-degrading enzyme genes, or both, respectively. Lines with arrows indicate activation, whereas barred lines indicate inhibition. This regulatory network is time-dependent. "+P" shows phosphorylation

(Li et al. 2015). The C-terminal residues 685–780 are considered as the transcriptional activation domain of ClrB, whereas the middle region (residues 173–684) represses cellulase gene expression (Gao et al. 2019). In addition, ClrB directly interacts with Tup1, thereby recruiting the co-activator complex Tup1-Cyc8. Tup1 then recruits the histone methyltransferase, LaeA, to modify the chromatin structure, thereby facilitating cellulolytic gene transcription (Zhang et al. 2022). Notably, expression of *clrB* is directly stimulated by another activator, CxrA; conversely, *cxrA* transcription is repressed by ClrB in the later stages of Avicel induction in *P. oxalicum* (Liao et al. 2019).

CxrA is considered to be a broad-spectrum activator, which, in the presence of Avicel, not only promotes the expression of major cellulase and xylanase genes, including *cbh1*, *eg1*, *eg2*, *bgl1*, and *xyn11A*, but also regulates the transcription of many regulatory genes, including *clrB*, *cxrB*, *cxrC*, *nsdD*, *cbh*, and *cxrD*, as well as cellodextrin transporter-encoding genes, *cdtC* and *cdtD*. Furthermore, in vitro EMSA demonstrated that CxrA can bind to the promoter regions of its target genes (Zhao et al. 2016; Yan et al. 2017; He et al. 2018; Li et al. 2021; Liao et al. 2019; Zhao et al. 2023b). Remarkably, the N-terminal region, $CxrA_{1–206}$, containing a DNA-binding domain ($CxrA_{17–58)}$ and a methylated arginine (R) at position 94, exhibits most of the regulatory functions of the full-length CxrA. Methylated R94 is essential for cellulase and xylanase production in *P. oxalicum* (Zhao et al. 2023c).

Regulation of xylanase gene expression involves the Zn2Cys6-type promoter, XlnR, also called Xyr1, or Xlr-1 in *T. reesei* and *Neurospora crassa*, respectively. In *P. oxalicum*, knockout of *xlnR* abolishes the expression of major xylanase genes, but only slightly reduces cellulase gene transcription (Li et al. 2015). The regulatory functions of XlnR are affected by the hydrophobicity of the alanine residue at position 871. The residues 351–694 function as the transcriptional activation domain of XlnR (Xia et al. 2022).

The main activator of amylase gene expression, AmyR, a homologous protein with COL26 in *N. crassa*, represses the expression of major cellulase genes in the presence of cellulose. AmyR can bind to the promoters of amylase genes, including *PoxGA15A* and *amy13A*, recruiting the histone acetyltransferase complex, Hat1-Hat2, through interaction with Hat2. Individual deletion of *hat1* and *hat2* upregulates amylase expression, thereby enhancing amylase production. Moreover, the complex Hat1-Hat2 can interact with corepressor Tup1-Cyc8, leading to downregulation of gene expression (Hu et al. 2023).

The TF, RsrA (formerly POX01907) specifically controls the transcription of primary amylase genes in the presence of starch, but does not influence cellulase and xylanase production under cellulose induction (Zhang et al. 2019). RsrA contains two SANT (**S**WI3, **A**DA2, **N**-CoR, and **T**FIIIB)-like domains, but with low sequence homology and different functions. SANT1 (residues 833-881) binds to a core DNA sequence, 5′-RHCDDGGD-3′ in the promoter regions of major amylase genes; the arginine (R) residue at position 866 is essential for DNA binding. SANT2 (residues 1086-1134) interacts with a putative 3-hydroxyisobutyryl-CoA hydrolase (POX_g08550) to block the phosphorylation of tyrosines Y1127 and Y1170, thereby downregulating RSDE and SSDE production. RsrA upregulates RSDE and SSDE biosynthesis by interacting with mediator subunit Med31. Moreover, the residues 1434-1730 have a transcriptional activation function, with D1508, W1509, and M1510 being essential for this interaction (Ning et al. 2023).

In addition, CAZyme biosynthesis is also regulated by other specific TFs, under induction by various carbon sources. For example, the HMG-box protein HmbB promotes cellulase and xylanase production in the presence of cellulose, but inhibits RSDE production in the presence of soluble corn starch (Xiong et al. 2018). The CENPB-type HTH domain protein, Cbh, regulates the expression of cellulase and xylanase on cellulose during submerged-state fermentation, but is inactive during solid-state fermentation (Li et al. 2021).

In addition to the TFs described above, many non-TFs have recently been discovered to be involved in PPDE biosynthesis by *P. oxalicum*. The translational elongation factor, eEF1A, interacts with CxrC to activate cellulase, xylanase, and amylase gene expression (Zhao et al. 2022c). G protein γ-subunit GNG-1 (Pang et al. 2021), MAPK PoxMK1 (Ma et al. 2021), PoxMKK1 (Ma et al. 2023), and glycogen synthase kinase-3β (GSK-3β) (Zhang et al. 2023) mediate cellulase, xylanase, and amylase gene expression, as well as that of known regulatory genes, such as *cxrB* and *clrB*. The AdoMet synthetase, PoSasA, upregulates cellulase gene expression (Hu et al. 2021b). The putative protein methyltransferase, Mtr23B, upregulates cellulase and amylase gene expression (Zhang et al. 2020).

In other *Penicillium* species, there is only one report, that Mig1, a protein homologous to CreA, represses cellulase gene expression in *Penicillium funiculosum* (Randhawa et al. 2018).

Breeding of *Penicillium* PPDE-hyperproducing strains

Despite great efforts to improve it, the production of PPDEs by *Penicillium* sp., specifically *P. oxalicum*, is still insufficient for large-scale biorefinery application. Rationally designed molecular breeding of *Penicillium* is therefore indispensable to improve PPDE production. Common breeding methods include screening for natural hyperproducing strains, physical and/or chemical mutagenesis,

and molecular breeding (Peterson and Nevalainen 2012). To date, many *Penicillium* sp. capable of PPDE secretion have been obtained, for example, *Penicillium polonicum* strain CCDCA10747, which secretes CMCase, avicelase, pectinase, mannanase, and xylanase during growth in liquid medium containing sugarcane bagasse as the carbon source. The highest production of endo-β-1,4-xylanase was obtained after 4 days of growth, reaching 2.24 U/mL, as well as CMCase and pectinase, each at 0.1 U/mL (De Camargo et al. 2022). Xylanase production by *Penicillium chrysogenum* was 1.08 U/mL and 0.94 U/mL in the presence of sugarcane straw and sugarcane bagasse, respectively (Ullah et al. 2019). *Penicillium pulvillorum* secretes cellulase, for example, 0.9 U/mL of FPase, in liquid medium containing Avicel as the carbon source (Marjamaa et al. 2013). *Penicillium citrinum* yielded maximum amylase production of 121.84 U/mg and FPase of 46.94 U/mg, from solid-state fermentation with wheat bran as substrate (Shyama and Shilpi 2014; Shruthi et al. 2020). *Penicillium subrubescens* produces an enzyme mixture with a similar composition and relative enzyme activities to *A. niger* with plant biomass as substrate. The *P. subrubescens* enzyme mixture has been applied to saccharify complex plant biomass, such as wheat bran and sugar beet pulp (Mäkelä et al. 2016). CMCase and endo-xylanase production by *Penicillium ochrochloron* RLS11 were 4.0 and 40.0 U/mg, respectively, when grown on steam-exploded sugarcane straw (Morgan et al. 2022). PPDE production by *P. oxalicum* strains HP7-1 (Zhang et al. 2014; Lin et al. 2011), Z1-3 (Jing et al. 2015), 114-2 (Liu et al. 2013a), and GXU20 (Lin et al. 2011) ranged from 0.5 to 55.1 U/mL (Table 1).

To obtain *Penicillium* strains with higher PPDE production, physical and chemical mutagenic methods have been employed. For example, *Penicillium janthinellum* NCIM 1171 was subjected to mutation by treatment with ethyl methyl sulfonate (EMS) for 24 h, followed by UV irradiation for 3 min, to obtain mutant NCIM1366, which could produce 0.83 U/mL of FPase, an increase of 28% as compared with the start strain (Sreeja-Raju et al. 2020). Using *P. oxalicum* HP7-1 as the starting strain, three rounds of Co^{60}-γ ray irradiation, combined with two rounds of EMS/ultraviolet (UV) treatment, produced mutant EU2106, with FPase production of 2.78 U/mL, 55% higher than that of HP7-1, when cultured on medium containing wheat bran plus Avicel (Zhao et al. 2016). Moreover, mutant JU-A10-T, with FPase production of 4.5 U/mL, eight times that of the starting strain, was derived from *P. oxalicum* strain 114-2 after multiple rounds of physical-chemical mutagenesis, including UV irradiation and nitrosoguanidine, and adaptation in medium containing spent ammonium sulfite liquor (Liu et al. 2013b). Similarly, mutant TE4-10 was obtained from *P. oxalicum* strain OX*PoxGA15A* (Wang et al. 2018) through multiple rounds of EMS, atmospheric and room-temperature plasma, and Co^{60}-γ ray irradiation mutagenesis, and produced 218.6 U/mL of RSDE, when cultured in medium containing Avicel plus wheat bran for 8 days, a 2.2-fold increase relative to the starting strain (Gu et al. 2020).

The fast development of genome sequencing and molecular manipulation techniques has promoted molecular breeding for improving PPDE production. PPDE biosynthesis is tightly regulated by many regulatory proteins, including TFs, that are potential targets for molecular breeding. For example, simultaneous knockout of two transcriptional repressor genes, *atf1* (Zhao et al. 2019) and *cxrC*, in *P. oxalicum* strain Δ*PoxKu70* (Zhao et al. 2016) significantly increased cellulase and xylanase production by 2.4–29.1-fold and 78.9–130.8%, respectively (Lin et al. 2021). In addition, the engineered strain GXUR001 was obtained by deletion of transcription suppressor, *cxrC*, and overexpression of activator gene, *amyR,* in the mutant TE4-10; its RSDE production reached 252.6 U/mL when cultured on medium containing Avicel plus wheat bran for 8 days, an increase of 15.6% relative to that of TE4-10 (Zhao et al. 2022a). A quadruple mutant, RE-27, was produced from *P. oxalicum* strain 114-2 by simultaneous deletion of transcriptional repressors, *bgl2* and *creA*, and overexpression of transcriptional activator gene, *clrB*; cellulase and xylanase production by the mutant RE-27 was 34.8%, 62.3% and 288.5% higher than that of the starting strain, respectively (Li et al. 2015). Another mutant, RE-8, generated by deletion of *creA* and overexpression of *clrB*, and cellulase genes, *cbh1* and *eg1*, increased cellulase and xylanase production by 3.9–30.6-fold (Gao et al. 2017).

In addition to transcription factor engineering, regulatory elements including promoters and signal peptides can also be modified to improve PPDE production. Screening the transcriptome and secretome of *P. oxalicum* strain HP7-1, combined with the use of a green fluorescent protein (GFP) reporter, found a strong promoter, pPoxEgCel5B, from the EG gene *POX01166*, and a strong signal peptide, spPoxGA15A from RSDE PoxGA15A, suitable for amylase production. The resulting engineered strain, OX*PoxGA15A*, had 3.4-fold higher RSDE production compared with the starting strain Δ*PoxKu70*, reaching 241.6 U/mL with raw cassava flour as substrate (Wang et al. 2018).

Strong synergy between gene transcription and translation is essential for highly efficient protein expression. A genetically engineered strain, Δ*cxrC*::*eEF1A*, in which the transcriptional repressor gene, *cxrC*, and the translation elongation factor gene, *eEF1A*, were deleted and overexpressed in the background strain Δ*PoxKu70*, respectively, had markedly increased cellulase, xylanase, and RSDE production. Notably, an in vitro GST-pulldown assay indicated that CxrC interacts with eEF1A (Zhao et al. 2022c).

Although the above reports indicate that PPDE production by *P. oxalicum* can be effectively increased by random mutagenesis and molecular breeding, the cost of

Table 1 List of *Penicillium* strains producing plant-polysaccharide-degrading enzymes obtained through screening from nature and artificial breeding

Penicillium	Parental strain	Characteristics or way of obtaining	Inducing carbon source	Yield of enzyme	References
Penicillium oxalicum HP7-1	Wild type	Isolated from Guangxi forestry soil	Wheat bran and Avicel; or soluble corn starch	FPase: 1.79 U/mL RSDE: 55.1 U/mL	Zhang et al. (2014), Zhao et al. (2016), Gu et al. (2020)
P. oxalicum Z1-3	Wild type	Isolated from Guangxi forestry soil	Wheat bran and Avicel	FPase: 2.74 U/mL	Jing et al. (2015)
P. oxalicum GXU20	Wild type	Isolated from Guangxi forestry soil	Soluble corn starch	RSDE: 20 U/mL	Lin et al. (2011)
P. oxalicum 114-2	Wild type	Isolated from soil in Jining, Shandong	Cellulose and wheat bran	FPase: ~ 0.5 U/mL	Liu et al. (2013a)
Penicillium polonicum CCDCA10747	Wild type	Isolated from Cerrado soil	Sugarcane bagasse	Xylanase: 2.243 U/mL, CMCase and pectinase: 0.1 U/mL	De Camargo et al. (2022)
Penicillium chrysogenum CCDCA10746	Wild type	Isolated from Brazilian Cerrado soil	Sugarcane straw; sugarcane bagasse	Xylanase: 1.081 U/mL; 0.940 U/mL	Ullah et al. (2019)
Penicillium pulvillorum TUB F-2220	Wild type	Isolated from Danube, Vac, Hungary soil	Avicel	FPase: 0.9 U/mL	Marjamaa et al. (2013)
Penicillium citrinum	Wild type	Isolated from India soil	Wheat bran	Amylase: 121.84 U/mg, FPase: 46.94 U/mg	Shyama and Shilpi (2014), Shruthi et al. (2020)
Penicillium ochrochloron RLS11	Wild type	Isolated from Brazil soil	Sugarcane straw	CMCase: 4.0 U/mg Xylanase: 40.0 U/mg	Morgan et al. (2022)
Penicillium janthinellum NCIM1366	*Penicillium janthinellum*NCIM 1171	Ethyl methyl sulfonate and UV irradiation	Cellulose	FPase: 0.83 U/mL	Sreeja-Raju et al. (2020)
P. oxalicum EU2106	HP7-1	γ-Irradiation and ethyl methanesulfonate/ultraviolet light mutagenesis	Sugarcane bagasse	FPase: 2.78 U/mL	Zhao et al. (2016)
P. oxalicum TE4-10	HP7-1	Six rounds of ethyl methanesulfonate and Co60-γ-ray mutagenesis	Soluble corn starch	RSDE: 218 U/mL	Zhao et al. (2022a)
P. oxalicum OX*PoxGA15A*	HP7-1	The gene *PoxGA15A* was overexpressed in Δ*PoxKu70* using the strong promoter pPoxEgCel5B and the strong signal peptide pPoxGA15A	Wheat bran and Avicel	RSDE: 241.6 U/mL	Wang et al. (2018)
P. oxalicum Δ*PoxAtf1*Δ*PoxCxrC*	HP7-1	Deletion of genes *PoxAtf1* and *PoxCxrC* in strain Δ*PoxKu70*	Wheat bran plus rice straw	FPase production increased 2.4 to 29.1 times Xylanase production increased 78.9–130.8%	Lin et al. (2021)
P. oxalicum Δ*cxrC*::*eEF1A*	HP7-1	Simultaneous deletion of gene *PoxCxrC* and overexpression of gene *eEF1A* in strain Δ*PoxKu70*	Avicel; or wheat bran and plus rice straw	Cellulase production increased 14.7–127.7% Xylanase production increased 31.7–217.8% RSDE production increased 55.4–314.6%	Zhao et al. (2022c)

Table 1 (continued)

Penicillium	Parental strain	Characteristics or way of obtaining	Inducing carbon source	Yield of enzyme	References
P. oxalicum GXUR001	HP7-1	Simultaneous deletion of gene *PoxCxrC* and overexpression of gene *PoxAmyR* in strain TE4-10	Raw starch	RSDE: 252.6 U/mL	Zhao et al. (2022a)
P. oxalicum RE-27	RE-10	Simultaneous deletion of genes *bgl2* and *creA*, and overexpression of gene *clrB* in strain RE-10	Cellulose and wheat bran	FPase: 8.85 U/mL, xylanase: 1341.97 U/mL, amylase: 125.07 U/mL	Li et al. (2015)
P. oxalicum JU-A10-T	114-2	Multiple rounds of random mutagenesis and genome shuffling	Cellulose and wheat bran	FPase: ~ 4.5 U/mL	Liu et al. (2013b)
P. oxalicum RE-8	114-2	Simultaneous deletion of genes *creA*, and overexpression of gene *clrB*, *cbh1*, *and eg1*	Wheat bran	Cellulase production increased 3.9–30.6 times, xylanase production increased 29.7 times	Gao et al. (2017)

FPase filter paper cellulase, *CMCase* carboxymethyl cellulase, *RSDE* raw-starch-degrading enzyme

PPDEs still does not meet the requirements for industrial application. However, it should be noted that the choice of breeding methods has been very limited, mainly using conventional mutagens, such as UV, Co^{60}, and EMS, and transcription factor engineering, compared with other industrial filamentous fungi, such as *T. reesei* and *Aspergillus niger*. In addition, the previously adopted genetic manipulation systems and screening strategies are time-consuming, specifically homologous recombination and hydrolysis zone-based high-throughput plating. Therefore, more modern breeding methods specifically suited to *Penicillium*, especially *P. oxalicum*, should be explored as soon as possible, such as CRISPR-Cas-mediated in vivo mutagenesis (Zimmermann et al. 2023), machine learning-guided protein engineering (Xu et al. 2023), and multiple omic-based molecular breeding. In addition, the popular screening methods such as droplet-based microfluidic high-throughput screening (He et al. 2019) and flow cytometry-based ultra-high throughput screening (Yang et al. 2022) should be considered.

A major challenge to the development of novel breeding methods for *Penicillium* is the limited knowledge of the regulatory mechanism of PPDE biosynthesis, which limits the applicable gene manipulation techniques and targets. Future research should focus on improving understanding of the regulatory mechanism of PPDE biosynthesis.

Summary and future prospects

The development and application of PPDEs will help to promote large-scale production and application of environmentally friendly and sustainable biological manufacturing. *Penicillium* has great potential for PPDE production because of its abundant PPDE gene resource and the high efficiency of PPDEs for plant polysaccharide hydrolysis. Many *Penicillium* regulatory factors involved in regulation of PPDE gene expression have been identified and several engineered hyperproducing PPDE strains have been obtained by manipulating these regulatory targets, in combination with random mutagenesis. Although some progress has been made towards understanding the biosynthetic mechanisms involved, as well as development and utilization of PPDEs in *Penicillium*, there is still some way to go, to achieve the ultimate aim of large-scale industrial application. Therefore, future studies should focus on (1) understanding the global regulatory mechanism of PPDE biosynthesis; (2) screening and identification of more regulatory elements at the genetic level; and (3) exploring and constructing highly efficient and practical breeding techniques and platforms.

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Declarations

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

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References

Alam MA, Kamlangdee N, Kelly JM (2017) The CreB deubiquitinating enzyme does not directly target the CreA repressor protein in *Aspergillus nidulans*. Curr Genet 63(4):647–667. https://doi.org/10.1007/s00294-016-0666-3

Behera BC, Sethi BK, Mishra RR, Dutta SK, Thatoi HN (2017) Microbial cellulases—diversity & biotechnology with reference to mangrove environment: a review. J Genet Eng Biotechnol 15(1):197–210. https://doi.org/10.1016/j.jgeb.2016.12.001

Chen Y, Dong L, Alam MA, Pardeshi L, Miao Z, Wang F, Tan K, Hynes MJ, Kelly JM, Wong KH (2021) Carbon catabolite repression governs diverse physiological processes and development in *Aspergillus nidulans*. mBio. 13(1):e0373421. https://doi.org/10.1128/mbio.03734-21

Cupertino FB, Virgilio S, Freitas FZ, Candido Tde S, Bertolini MC (2015) Regulation of glycogen metabolism by the CRE-1, RCO-1 and RCM-1 proteins in *Neurospora crassa*. The role of CRE-1 as the central transcriptional regulator. Fungal Genet Biol FG & B 77:82–94. https://doi.org/10.1016/j.fgb.2015.03.011

De Camargo BR, Takematsu HM, Ticona ARP, da Silva LA, Silva FL, Quirino BF, Hamann PRV, Noronha EF (2022) *Penicillium polonicum* a new isolate obtained from Cerrado soil as a source of carbohydrate-active enzymes produced in response to sugarcane bagasse. 3 Biotech 12(12):348. https://doi.org/10.1007/s13205-022-03405-x

Dharma Patria R, Rehman S, Vuppaladadiyam AK, Wang H, Lin CSK, Antunes E, Leu SY (2022) Bioconversion of food and lignocellulosic wastes employing sugar platform: a review of enzymatic hydrolysis and kinetics. Bioresour Technol 352:127083. https://doi.org/10.1016/j.biortech.2022.127083

Fang H, Zhao R, Li C, Zhao C (2019b) Simultaneous enhancement of the beta-exo synergism and exo-exo synergism in *Trichoderma reesei* cellulase to increase the cellulose degrading capability. Microb Cell Fact 18(1):9. https://doi.org/10.1186/s12934-019-1060-x

Fang W, Xue S, Deng P, Zhang X, Wang X, Xiao Y, Fang Z (2019a) AmyZ1: a novel α-amylase from marine bacterium *Pontibacillus sp*. ZY with high activity toward raw starches. Biotechnol Biofuels 12:95. https://doi.org/10.1186/s13068-019-1432-9

Gao L, Li Z, Xia C, Qu Y, Liu M, Yang P, Yu L, Song X (2017) Combining manipulation of transcription factors and overexpression of the target genes to enhance lignocellulolytic enzyme production in *Penicillium oxalicum*. Biotechnol Biofuels 10:100. https://doi.org/10.1186/s13068-017-0783-3

Gao L, Xu Y, Song X, Li S, Xia C, Xu J, Qin Y, Liu G, Qu Y (2019) Deletion of the middle region of the transcription factor ClrB in *Penicillium oxalicum* enables cellulase production in the presence of glucose. J Biol Chem 294(49):18685–18697. https://doi.org/10.1074/jbc.RA119.010863

Gu LS, Tan MZ, Li SH, Zhang T, Zhang QQ, Li CX, Luo XM, Feng JX, Zhao S (2020) ARTP/EMS-combined multiple mutagenesis efficiently improved production of raw starch-degrading enzymes in *Penicillium oxalicum* and characterization of the enzyme-hyperproducing mutant. Biotechnol Biofuels 13(1):187. https://doi.org/10.1186/s13068-020-01826-5

Guo X, An Y, Liu F, Lu F, Wang B (2022) Lytic polysaccharide monooxygenase—a new driving force for lignocellulosic biomass degradation. Bioresour Technol 362:127803. https://doi.org/10.1016/j.biortech.2022.127803

He QP, Zhao S, Wang JX, Li CX, Yan YS, Wang L, Liao LS, Feng JX (2018) Transcription factor NsdD regulates the expression of genes involved in plant biomass-degrading enzymes, conidiation, and pigment biosynthesis in *Penicillium oxalicum*. Appl Environ Microbiol 84(18):e01039–e01018. https://doi.org/10.1128/AEM.01039-18

He R, Ding R, Heyman JA, Zhang D, Tu R (2019) Ultra-high-throughput picoliter-droplet microfluidics screening of the industrial cellulase-producing filamentous fungus *Trichoderma reesei*. J Ind Microbiol Biotechnol 46(11):1603–1610. https://doi.org/10.1007/s10295-019-02221-2

Hemati A, Nazari M, Asgari Lajayer B, Smith DL, Astatkie T (2022) Lignocellulosics in plant cell wall and their potential biological degradation. Folia Microbiol (Praha) 67(5):671–681. https://doi.org/10.1007/s12223-022-00974-5

Hu Y, Li M, Liu Z, Song X, Qu Y, Qin Y (2021a) Carbon catabolite repression involves physical interaction of the transcription factor CRE1/CreA and the Tup1-Cyc8 complex in *Penicillium oxalicum* and *Trichoderma reesei*. Biotechnol Biofuels 14(1):244. https://doi.org/10.1186/s13068-021-02092-9

Hu Y, Liu Z, Xu S, Zhao Q, Liu G, Song X, Qu Y, Qin Y (2023) The interaction between the histone acetyltransferase complex Hat1-Hat2 and transcription factor AmyR provides a molecular brake to regulate amylase gene expression. Mol Microbiol 119(4):471–491. https://doi.org/10.1111/mmi.15036

Hu Y, Zhao K, Qu Y, Song X, Zhao J, Qin Y (2021b) *Penicillium oxalicum* S-adenosylmethionine synthetase is essential for the viability of fungal cells and the expression of genes encoding cellulolytic enzymes. Fungal Biol 125(1):1–11. https://doi.org/10.1016/j.funbio.2020.09.004

Jing L, Zhao S, Xue JL, Zhang Z, Yang Q, Xian L, Feng JX (2015) Isolation and characterization of a novel *Penicillium oxalicum* strain Z1-3 with enhanced cellobiohydrolase production using cellulase-hydrolyzed sugarcane bagasse as carbon source. Ind Crop Prod 77:666–675. https://doi.org/10.1016/j.indcrop.2015.09.052

Lenz AR, Balbinot E, de Abreu FP, de Oliveira NS, Fontana RC, de Avila E, Silva S, Park MS, Lim YW, Houbraken J, Camassola M, Dillon AJP (2022b) Taxonomy, comparative genomics and evolutionary insights of *Penicillium ucsense*: a novel species in series Oxalica. Antonie Van Leeuwenhoek 115(8):1009–1029. https://doi.org/10.1007/s10482-022-01746-4

Lenz AR, Balbinot E, Souza de Oliveira N, Abreu FP, Casa PL, Camassola M, Perez-Rueda E, de Avila E, Silva S, Dillon AJP (2022a) Analysis of carbohydrate-active enzymes and sugar transporters in *Penicillium echinulatum*: a genome-wide comparative study of the fungal lignocellulolytic system. Gene. 822:146345. https://doi.org/10.1016/j.gene.2022.146345

Li CX, Liao LS, Wan XD, Zhang FF, Zhang T, Luo XM, Zhao S, Feng JX (2021) PoxCbh, a novel CENPB-type HTH domain protein, regulates cellulase and xylanase gene expression in *Penicillium oxalicum*. Mol Microbiol 116(1):140–153. https://doi.org/10.1111/mmi.14696

Li CX, Liu L, Zhang T, Luo XM, Feng JX, Zhao S (2022b) Three-dimensional genome map of the filamentous fungus *Penicillium oxalicum*. Microbiol Spectr 10(3):e0212121. https://doi.org/10.1128/spectrum.02121-21

Li Y, Song W, Han X, Wang Y, Rao S, Zhang Q, Zhou J, Li J, Liu S, Du G (2022a) Recent progress in key lignocellulosic enzymes: enzyme discovery, molecular modifications, production, and enzymatic biomass saccharification. Bioresour Technol 363:127986. https://doi.org/10.1016/j.biortech.2022.127986

Li Z, Liu G, Qu Y (2017) Improvement of cellulolytic enzyme production and performance by rational designing expression regulatory network and enzyme system composition. Bioresour Technol 245(Pt B):1718–1726. https://doi.org/10.1016/j.biortech.2017.06.120

Li Z, Yao G, Wu R, Gao L, Kan Q, Liu M, Yang P, Liu G, Qin Y, Song X, Zhong Y, Fang X, Qu Y (2015) Synergistic and dose-controlled regulation of cellulase gene expression in *Penicillium oxalicum*. PLoS Genet 11(9):e1005509. https://doi.org/10.1371/journal.pgen.1005509

Liao LS, Li CX, Zhang FF, Yan YS, Luo XM, Zhao S, Feng JX (2019) How an essential Zn2Cys6 transcription factor PoxCxrA regulates cellulase gene expression in ascomycete fungi? Biotechnol Biofuels 12:105. https://doi.org/10.1186/s13068-019-1444-5

Lin HJ, Xian L, Zhang QJ, Luo XM, Xu QS, Yang Q, Duan CJ, Liu JL, Tang JL, Feng JX (2011) Production of raw cassava starch-degrading enzyme by *Penicillium* and its use in conversion of raw cassava flour to ethanol. J Ind Microbiol Biotechnol 38(6):733–742. https://doi.org/10.1007/s10295-010-0910-7

Lin YY, Zhao S, Lin X, Zhang T, Li CX, Luo XM, Feng JX (2021) Improvement of cellulase and xylanase production in *Penicillium oxalicum* under solid-state fermentation by flippase recombination enzyme/ recognition target-mediated genetic engineering of transcription repressors. Bioresour Technol 337:125366. https://doi.org/10.1016/j.biortech.2021.125366

Liu G, Zhang L, Qin Y, Zou G, Li Z, Yan X, Wei X, Chen M, Chen L, Zheng K, Zhang J, Ma L, Li J, Liu R, Xu H, Bao X, Fang X, Wang L, Zhong Y et al (2013b) Long-term strain improvements accumulate mutations in regulatory elements responsible for hyper-production of cellulolytic enzymes. Sci Rep 3:1569. https://doi.org/10.1038/srep01569

Liu G, Zhang L, Wei X, Zou G, Qin Y, Ma L, Li J, Zheng H, Wang S, Wang C, Xun L, Zhao GP, Zhou Z, Qu Y (2013a) Genomic and secretomic analyses reveal unique features of the lignocellulolytic enzyme system of *Penicillium decumbens*. PloS One 8(2):e55185. https://doi.org/10.1371/journal.pone.0055185

Liu HQ, Feng Y, Zhao DQ, Jiang JX (2012) Evaluation of cellulases produced from four fungi cultured on furfural residues and microcrystalline cellulose. Biodegradation. 23(3):465–472. https://doi.org/10.1007/s10532-011-9525-6

Long L, Wang W, Liu Z, Lin Y, Wang J, Lin Q, Ding S (2023) Insights into the capability of the lignocellulolytic enzymes of *Penicillium parvum* 4-14 to saccharify corn bran after alkaline hydrogen peroxide pretreatment. Biotechnol Biofuels Bioprod 16(1):79. https://doi.org/10.1186/s13068-023-02319-x

Ma B, Luo XM, Zhao S, Feng JX (2023) Protein kinase PoxMKK1 regulates plant-polysaccharide-degrading enzyme biosynthesis, mycelial growth and conidiation in *Penicillium oxalicum*. J Fungi 9(4):397. https://doi.org/10.3390/jof9040397

Ma B, Ning YN, Li CX, Tian D, Guo H, Pang XM, Luo XM, Zhao S, Feng JX (2021) A mitogen-activated protein kinase PoxMK1 mediates regulation of the production of plant-biomass-degrading enzymes, vegetative growth, and pigment biosynthesis in *Penicillium oxalicum*. Appl Microbiol Biotechnol 105(2):661–678. https://doi.org/10.1007/s00253-020-11020-0

Mäkelä MR, Mansouri S, Wiebenga A, Rytioja J, de Vries RP, Hildén KS (2016) *Penicillium subrubescens* is a promising alternative for *Aspergillus niger* in enzymatic plant biomass saccharification. N Biotechnol 33(6):834–841. https://doi.org/10.1016/j.nbt.2016.07.014

Marjamaa K, Toth K, Bromann PA, Szakacs G, Kruus K (2013) Novel *Penicillium* cellulases for total hydrolysis of lignocellulosics. Enzyme Microb Technol 52(6-7):358–369. https://doi.org/10.1016/j.enzmictec.2013.03.003

Mendonça M, Barroca M, Collins T (2023) Endo-1,4-β-xylanase-containing glycoside hydrolase families: characteristics, singularities and similarities. Biotechnol Adv 65:108148. https://doi.org/10.1016/j.biotechadv.2023.108148

Monclaro AV, Gorgulho Silva CO, Gomes HAR, Moreira LRS, Filho EXF (2022) The enzyme interactome concept in filamentous fungi linked to biomass valorization. Bioresour Technol 344(Pt A):126200. https://doi.org/10.1016/j.biortech.2021.126200

Morgan T, Falkoski DL, Tavares MP, Oliveira MB, Guimarães VM, de Oliveira Mendes TA (2022) *Penicillium Ochrochloron* RLS11 secretome containing carbohydrate-active enzymes improves commercial enzyme mixtures during sugarcane straw saccharification. Appl Biochem Biotechnol 194(7):2946–2967. https://doi.org/10.1007/s12010-022-03898-5

Ning P, Yang G, Hu L, Sun J, Shi L, Zhou Y, Wang Z, Yang J (2021) Recent advances in the valorization of plant biomass. Biotechnol Biofuels 14(1):102. https://doi.org/10.1186/s13068-021-01949-3

Ning YN, Tian D, Tan ML, Luo XM, Zhao S, Feng JX (2023) Regulation of fungal raw-starch-degrading enzyme production depends on transcription factor phosphorylation and recruitment of the Mediator complex. Commun Biol 6(1):1032. https://doi.org/10.1038/s42003-023-05404-x

Pang XM, Tian D, Zhang T, Liao LS, Li CX, Luo XM, Feng JX, Zhao S (2021) G protein γ subunit modulates expression of plant-biomass-degrading enzyme genes and mycelial-development-related genes in *Penicillium oxalicum*. Appl Microbiol Biotechnol 105(11):4675–4691. https://doi.org/10.1007/s00253-021-11370-3

Passos DDF, Pereira N, Castro AMD (2018) A comparative review of recent advances in cellulases production by *Aspergillus*, *Penicillium* and *Trichoderma* strains and their use for lignocellulose deconstruction. Curr Opin Green Sustain Chem 14:60–66. https://doi.org/10.1016/j.cogsc.2018.06.003

Petersen C, Sørensen T, Nielsen MR, Sondergaard TE, Sørensen JL, Fitzpatrick DA, Frisvad JC, Nielsen KL (2023) Comparative genomic study of the *Penicillium* genus elucidates a diverse pangenome and 15 lateral gene transfer events. IMA Fungus 14(1):3. https://doi.org/10.1186/s43008-023-00108-7

Peterson R, Nevalainen H (2012) *Trichoderma reesei* RUT-C30--thirty years of strain improvement. Microbiology (Reading) 158(Pt 1):58–68. https://doi.org/10.1099/mic.0.054031-0

Pham HM, Le DT, Le LT, Chu PTM, Tran LH, Pham TT, Nguyen HM, Luu TT, Hoang H, Chu HH (2023) A highly quality genome sequence of *Penicillium oxalicum* species isolated from the root of Ixora chinensis in Vietnam. G3 (Bethesda) 13(2):jkac300. https://doi.org/10.1093/g3journal/jkac300

Randhawa A, Ogunyewo OA, Eqbal D, Gupta M, Yazdani SS (2018) Disruption of zinc finger DNA binding domain in catabolite repressor Mig1 increases growth rate, hyphal branching, and cellulase expression in hypercellulolytic fungus *Penicillium funiculosum* NCIM1228. Biotechnol Biofuels 11:15. https://doi.org/10.1186/s13068-018-1011-5

Ribeiro LFC, Chelius C, Boppidi KR, Naik NS, Hossain S, Ramsey JJJ, Kumar J, Ribeiro LF, Ostermeier M, Tran B, Ah Goo Y, de Assis LJ, Ulas M, Bayram O, Goldman GH, Lincoln S, Srivastava R,

Harris SD, Marten MR (2019) Comprehensive analysis of *Aspergillus nidulans* PKA phosphorylome identifies a novel mode of CreA regulation. mBio. 10(2):e02825–e02818. https://doi.org/10.1128/mBio.02825-18

Shruthi BR, Achur RNH, Nayaka Boramuthi T (2020) Optimized solid-state fermentation medium enhances the multienzymes production from *Penicillium citrinum* and *Aspergillus clavatus*. Curr Microbiol 77(9):2192–2206. https://doi.org/10.1007/s00284-020-02036-w

Shyama PS, Shilpi G (2014) Optimization of xylanase production by *Penicillium citrinum* xym2 and application in saccharification of agro-residues. Biocatal Biotransformation 3(4):188–196. https://doi.org/10.1016/j.bcab.2014.03.003

Sreeja-Raju A, Christopher M, Kooloth-Valappil P, Kuni-Parambil R, Gokhale DV, Sankar M, Abraham A, Pandey A, Sukumaran RK (2020) *Penicillium janthinellum* NCIM1366 shows improved biomass hydrolysis and a larger number of CAZymes with higher induction levels over *Trichoderma reesei* RUT-C30. Biotechnol Biofuels 13(1):196. https://doi.org/10.1186/s13068-020-01830-9

Sukumaran RK, Christopher M, Kooloth-Valappil P, Sreeja-Raju A, Mathew RM, Sankar M, Puthiyamadam A, Adarsh VP, Aswathi A, Rebinro V, Abraham A, Pandey A (2021) Addressing challenges in production of cellulases for biomass hydrolysis: Targeted interventions into the genetics of cellulase producing fungi. Bioresour Technol 329:124746. https://doi.org/10.1016/j.biortech.2021.124746

Sun H, Zhao P, Ge X, Xia Y, Hao Z, Liu J, Peng M (2010) Recent advances in microbial raw starch degrading enzymes. Appl Biochem Biotechnol 160(4):988–1003. https://doi.org/10.1007/s12010-009-8579-y

Sun HY, Zhao PJ, Peng M (2008) Application of maltitol to improve production of raw starch digesting glucoamylase by *Aspergillus niger* F-08. World J Microbiol Biotechnol 24(11):2613–2618. https://doi.org/10.1007/s11274-008-9785-4

Ullah SF, Souza AA, Hamann PRV, Ticona ARP, Oliveira GM, Barbosa JARG, Freitas SM, Noronha EF (2019) Structural and functional characterization of xylanase purified from *Penicillium chrysogenum* produced in response to raw agricultural waste. Int J Biol Macromol 127:385–395. https://doi.org/10.1016/j.ijbiomac.2019.01.057

Vaishnav N, Singh A, Adsul M, Dixit P, Sandhu SK, Mathur A, Puri SK, Singhania RR (2018) *Penicillium*: the next emerging champion for cellulase production. Bioresource Technol Rep 2018(2):131–140. https://doi.org/10.1016/j.biteb.2018.04.003

Wang BT, Hu S, Yu XY, Jin L, Zhu YJ, Jin FJ (2020a) Studies of cellulose and starch utilization and the regulatory mechanisms of related enzymes in fungi. Polymers (Basel) 12(3):530. https://doi.org/10.3390/polym12030530

Wang K, Zhang N, Pearce R, Yi S, Zhao X (2021) Comparative secretomics analysis reveals the major components of *Penicillium oxalicum* 16 and *Trichoderma reesei* RUT-C30. Microorganisms. 9(10):2042. https://doi.org/10.3390/microorganisms9102042

Wang L, Zhao S, Chen XX, Deng QP, Li CX, Feng JX (2018) Secretory overproduction of a raw starch-degrading glucoamylase in *Penicillium oxalicum* using strong promoter and signal peptide. Appl Microbiol Biotechnol 102(21):9291–9301. https://doi.org/10.1007/s00253-018-9307-8

Wang Q, Zhong C, Xiao H (2020b) Genetic engineering of filamentous fungi for efficient protein expression and secretion. Front Bioeng Biotechnol 8:293. https://doi.org/10.3389/fbioe.2020.00293

Wu G, Jurick Ii WM, Lichtner FJ, Peng H, Yin G, Gaskins VL, Yin Y, Hua SS, Peter KA, Bennett JW (2019) Whole-genome comparisons of *Penicillium* spp. reveals secondary metabolic gene clusters and candidate genes associated with fungal aggressiveness during apple fruit decay. PeerJ. 7:e6170. https://doi.org/10.7717/peerj.6170

Xia C, Gao L, Li Z, Liu G, Song X (2022) Functional analysis of the transcriptional activator XlnR of *Penicillium oxalicum*. J Appl Microbiol 132(2):1112–1120. https://doi.org/10.1111/jam.15276

Xiong YR, Zhao S, Fu LH, Liao XZ, Li CX, Yan YS, Liao LS, Feng JX (2018) Characterization of novel roles of a HMG-box protein PoxHmbB in biomass-degrading enzyme production by *Penicillium oxalicum*. Appl Microbiol Biotechnol 102(8):3739–3753. https://doi.org/10.1007/s00253-018-8867-y

Xu QS, Yan YS, Feng JX (2016) Efficient hydrolysis of raw starch and ethanol fermentation: a novel raw starch-digesting glucoamylase from *Penicillium oxalicum*. Biotechnol Biofuels 9:216. https://doi.org/10.1186/s13068-016-0636-5

Xu S, Gao S, An Y (2023) Research progress of engineering microbial cell factories for pigment production. Biotechnol Adv 65:108150. https://doi.org/10.1016/j.biotechadv.2023.108150

Yan YS, Zhao S, Liao LS, He QP, Xiong YR, Wang L, Li CX, Feng JX (2017) Transcriptomic profiling and genetic analyses reveal novel key regulators of cellulase and xylanase gene expression in *Penicillium oxalicum*. Biotechnol Biofuels 10:279. https://doi.org/10.1186/s13068-017-0966-y

Yang YJ, Liu Y, Liu DD, Guo WZ, Wang LX, Wang XJ, Lv HX, Yang Y, Liu Q, Tian CG (2022) Development of a flow cytometry-based plating-free system for strain engineering in industrial fungi. Appl Microbiol Biotechnol 106(2):713–727. https://doi.org/10.1007/s00253-021-11733-w

Zhang MY, Zhao S, Ning YN, Fu LH, Li CX, Wang Q, You R, Wang CY, Xu HN, Luo XM, Feng JX (2019) Identification of an essential regulator controlling the production of raw-starch-digesting glucoamylase in *Penicillium oxalicum*. Biotechnol Biofuels 12:7. https://doi.org/10.1186/s13068-018-1345-z

Zhang T, Li HZ, Li WT, Tian D, Ning YN, Liang X, Tan J, Zhao YH, Luo XM, Feng JX, Zhao S (2023) Kinase POGSK-3β modulates fungal plant polysaccharide-degrading enzyme production and development. Appl Microbiol Biotechnol 107(11):3605–3620. https://doi.org/10.1007/s00253-023-12548-7

Zhang T, Mai RM, Fang QQ, Ou JF, Mo LX, Tian D, Li CX, Gu LS, Luo XM, Feng JX (2021) Zhao S. Regulatory function of the novel transcription factor CxrC in *Penicillium oxalicum*. Mol Microbiol 116(6):1512–1532. https://doi.org/10.1111/mmi.14843

Zhang X, Hu Y, Liu G, Liu M, Li Z, Zhao J, Song X, Zhong Y, Qu Y, Wang L, Qin Y (2022) The complex Tup1-Cyc8 bridges transcription factor ClrB and putative histone methyltransferase LaeA to activate the expression of cellulolytic genes. Mol Microbiol 117(5):1002–1022. https://doi.org/10.1111/mmi.14885

Zhang X, Li M, Zhu Y, Yang L, Li Y, Qu J, Wang L, Zhao J, Qu Y, Qin Y (2020) *Penicillium oxalicum* putative methyltransferase Mtr23B has similarities and differences with LaeA in regulating conidium development and glycoside hydrolase gene expression. Fungal Genet Biol 143:103445. https://doi.org/10.1016/j.fgb.2020.103445

Zhang Z, Liu JL, Lan JY, Duan CJ, Ma QS, Feng JX (2014) Predominance of *Trichoderma* and *Penicillium* in cellulolytic aerobic filamentous fungi from subtropical and tropical forests in China, and their use in finding highly efficient β-glucosidase. Biotechnol Biofuels 7:107. https://doi.org/10.1186/1754-6834-7-107

Zhao S, Liao XZ, Wang JX, Ning YN, Li CX, Liao LS, Liu Q, Jiang Q, Gu LS, Fu LH, Yan YS, Xiong YR, He QP, Su LH, Duan CJ, Luo XM, Feng JX (2019) Transcription factor Atf1 regulates expression of cellulase and xylanase genes during solid-state fermentation of *Ascomycetes*. Appl Environ Microbiol 85(24):e01226–e01219. https://doi.org/10.1128/AEM.01226-19

Zhao S, Mai RM, Zhang T, Feng XZ, Li WT, Wang WX, Luo XM, Feng JX (2022c) Simultaneous manipulation of transcriptional regulator CxrC and translational elongation factor eEF1A enhances the production of plant-biomass-degrading enzymes of

Penicillium oxalicum. Bioresour Technol 351:127058. https://doi.org/10.1016/j.biortech.2022.127058

Zhao S, Mo LX, Li WT, Jiang LL, Meng YY, Ou JF, Liao LS, Yan YS, Luo XM, Feng JX (2023c) Arginine methyltransferases PRMT2 and PRMT3 are essential for biosynthesis of plant-polysaccharide-degrading enzymes in *Penicillium oxalicum*. PLoS Genet 19(7):e1010867. https://doi.org/10.1371/journal.pgen.1010867

Zhao S, Tan MZ, Wang RX, Ye FT, Chen YP, Luo XM, Feng JX (2022a) Combination of genetic engineering and random mutagenesis for improving production of raw-starch-degrading enzymes in *Penicillium oxalicum*. Microb Cell Fact 21(1):272. https://doi.org/10.1186/s12934-022-01997-w

Zhao S, Wang JX, Hou R, Ning YN, Chen ZX, Liu Q, Luo XM, Feng JX (2023b) Novel transcription factor CXRD regulates cellulase and xylanase biosynthesis in *Penicillium oxalicum* under solid-state fermentation. Appl Environ Microbiol:e0036023. Advance online publication. https://doi.org/10.1128/aem.00360-23

Zhao S, Wang ZB, Wang YZ, Yang PY, Luo XM, Wu AM, Feng JX (2023a) Sustainable coproduction of xylooligosaccharide, single-cell protein and lignin-adsorbent through whole components' utilization of sugarcane bagasse with high solid loading. Sep Purif Technol 308:122916. https://doi.org/10.1016/j.seppur.2022.122916

Zhao S, Xiang B, Yang L, Chen J, Zhu C, Chen Y, Cui J, Hu S, Hu Y (2022b) Genetic modifications of critical regulators provide new insights into regulation modes of raw-starch-digesting enzyme expression in *Penicillium*. Biotechnol Biofuels Bioprod 15(1):62. https://doi.org/10.1186/s13068-022-02162-6

Zhao S, Yan YS, He QP, Yang L, Yin X, Li CX, Mao LC, Liao LS, Huang JQ, Xie SB, Nong QD, Zhang Z, Jing L, Xiong YR, Duan CJ, Liu JL, Feng JX (2016) Comparative genomic, transcriptomic and secretomic profiling of *Penicillium oxalicum* HP7-1 and its cellulase and xylanase hyper-producing mutant EU2106, and identification of two novel regulatory genes of cellulase and xylanase gene expression. Biotechnol Biofuels 9:203. https://doi.org/10.1186/s13068-016-0616-9

Zhao S, Zhang GL, Chen C, Yang Q, Luo XM, Wang ZB, Wu AM, Feng JX (2021) A combination of mild chemical pre-treatment and enzymatic hydrolysis efficiently produces xylooligosaccharides from sugarcane bagasse. J Clean Prod 291:125972. https://doi.org/10.1016/j.jclepro.2021.125972

Zimmermann A, Prieto-Vivas JE, Cautereels C, Gorkovskiy A, Steensels J, Van de Peer Y, Verstrepen KJ (2023) A Cas3-base editing tool for targetable in vivo mutagenesis. Nat Commun 14(1):3389. https://doi.org/10.1038/s41467-023-39087-z