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Original Research Article

Global regulation of fungal secondary metabolism in *Trichoderma reesei* by the transcription factor Ypr1, as revealed by transcriptome analysis

Jie Yang, Jia-Xiang Li, Fei Zhang, Xin-Qing Zhao*

State Key Laboratory of Microbial Metabolism, Joint International Research Laboratory of Metabolic & Developmental Sciences, School of Life Sciences and Biotechnology, Shanghai Jiao Tong University, Shanghai 200240, China

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A B S T R A C T

Trichoderma reesei Rut-C-30 is a well-known robust producer of cellulolytic enzymes, which are used to degrade lignocellulosic biomass for the sustainable production of biofuels and biochemicals. However, studies of its secondary metabolism and regulation remain scarce. Ypr1 was previously described as a regulator of the biosynthesis of the yellow pigment sorbicillin (a bioactive agent with great pharmaceutical interest) in *T. reesei* and several other fungi. However, the manner in which this regulator affects global gene transcription has not been explored. In this study, we report the effect of Ypr1 on the regulation of both the secondary and primary metabolism of *T. reesei* Rut-C30. A global gene transcription profile was obtained using a comparative transcriptomic analysis of the wild-type strain *T. reesei* Rut-C-30 and its *ypr1* deletion mutant. The results of this analysis suggest that, in addition to its role in regulating sorbicillin and the major extracellular (hemi)cellulases, Ypr1 also affects the transcription of genes encoding several other secondary metabolites. Although the primary metabolism of *T. reesei* Δ*ypr1* became less active compared with that of *T. reesei* Rut-C-30, several gene clusters involved in its secondary metabolism were activated, such as the gene clusters for the biosynthesis of specific polyketides and non-ribosomal peptides, together with the “sorbicillinoid–cellulase” super cluster, indicating that specific secondary metabolites and cellulases may be co-regulated in *T. reesei* Rut-C-30. The results presented in this study may benefit the development of genetic engineering strategies for the production of sorbicillin by *T. reesei* Rut-C-30, and provide insights for enhancing sorbicillin production in other filamentous fungal producers.

1. Introduction

Secondary metabolites are a very heterogeneous chemical group of low-molecular-weight compounds, many of which have important bioactivities and act as antimicrobials, antioxidants, pigments, hormones, and metal chelators. The biosynthesis of secondary metabolites can be classified into various types, including polyketides, non-ribosomal peptides, terpenes, and shikimic-acid-derived compounds; however, many compounds are hybrids of these classes [1]. The secondary metabolites of fungal producers are also receiving interest for their potential applications in medicine and feed industries, in addition to their roles in biocontrol [2]. These metabolites are usually produced in a family form of related compounds during a limited phase of the cell cycle or a specific phase of morphological differentiation, and play a survival role by supporting decomposition competition, symbiosis, and self-protection [3].

Trichoderma reesei (*Hypocrea jecorina*) is commonly used in the industrial production of cellulase, hemicellulase, and recombinant proteins, which have received widespread attention for the biorefinery of lignocellulosic biomass [4]. Moreover, various secondary metabolites are also produced by *T. reesei* that prevent the growth of competing microorganisms, which also enhance its biocontrol activity, thus benefiting plants in the defense against pathogenic fungi [5]. For example, paracelsins containing a high level of uncommon amino acids (such as alpha-aminoisobutyric acid (Aib) and isovaline (Iva)), and linear peptides of peptaibol non-ribosomal peptides (peptaibiotics), account for the antimicrobial activity of *T. reesei* [5]. Several other non-ribosomal peptides, such as siderophores, and polyketide compounds, such as aurofusarin and bikaverin, are probably precursors of the green pigment (melanin) present in the conidia of *T. reesei* [6]. The relationship between cellulase production and the accumulation of secondary metabolites has been examined; however, the manner in which the primary metabolism and secondary metabolisms are coordinated in *T. reesei* remains unclear [7].

“Sorbicillinoids,” which are commonly known as yellow pigment, are structurally polyketides belonging to a large class of hexaketide metabolites [8,9]. Both marine and terrestrial ascomycetes can produce and secrete yellow pigments, including *Trichoderma, Aspergillus*, and *Peni-*

* Corresponding author.
E-mail address: xqzhao@sjtu.edu.cn (X.-Q. Zhao).

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cillium [10–12]. Most of these pigment compounds have typical C1–C6 sorbitan lipid side chains and bicyclic or tricyclic skeletons, which are very complex and highly oxidized, and have a variety of biological activities, including anti-cancer, antioxidant, anti-viral, and antibacterial functions, thus exhibiting broad application prospects in the agricultural, pharmaceutical, and food industries [9].

Among the 30 secondary metabolite gene clusters included in the *T. reesei* genome, the yellow-pigment-producing polyketide gene cluster has been resolved, and the main regulators (Ypr1 and Ypr2) of the synthesis of the sorbicillinoid gene cluster have been revealed [13]. Ypr2 is an important regulator of the balance between carbon metabolism and secondary metabolism under cellulose induction and dark conditions in *T. reesei*; moreover, it can negatively regulate the expression of Ypr1 [13,14]. Among the regulators that mediate the expression of *sor1, sor2, sor3*, and *sor4* during the synthesis of the yellow pigment, hyperproduction of this pigment can be achieved via the overexpression of Ypr1 [13,15]. In addition, it was shown that hyperproduction of yellow pigment via Ypr1 can cause defects in mycelial growth, conidiation, cell-wall integrity, stress tolerance, and even extracellular cellobiohydrolase activity [15]. Although the deletion of *ypr1* hardly disturbs mycelial growth and cellulase production, conidiation, cell-wall integrity, and stress tolerance are enhanced in *T. reesei* QM9414 [15]. However, in addition to its role in targeting the sorbicillinoid PKS_11 cluster, the global regulatory functions of Ypr1 remain unclear.

In our previous study, we reported that overexpression of *ypr1* (*TrC30_93,861*) in the cellulase-hyperproduction strain *T. reesei* Rut-C30 reduced sporulation and mycelium growth significantly [16]. Although the deletion of *ypr1* did not affect cellulase activity, the transcription of cellulase-related genes was affected [16]. To date, studies of yellow pigment production in *T. reesei* focused exclusively on single gene clusters and specific regulators, whereas the manner in which global gene expression and metabolism affect the production of the yellow pigment remains unclear. Considering that the biosynthesis of secondary metabolites may be interconnected, and that metabolic engineering of primary metabolism also contributes to the production of useful secondary metabolites [17], it is of great interest to test whether *ypr1* also affects competing pathways.

In this study, the effect of *ypr1* deletion on global gene transcription in *T. reesei* Rut-C-30 was assessed using a comparative transcriptomic analysis. We found that loss of *ypr1* affected not only the transcription of genes involved in the production of cellulase and the yellow pigment, but also several other gene clusters. Our results provide insights regarding the cross-regulation of different secondary metabolites in *T. reesei* Rut-C-30, which may promote the development of engineering strategies for the production of useful fungal secondary metabolites.

2. Materials and methods

2.1. 2.1 Vegetative growth, conidiation, and sorbicillinoid production analyses

The deletion mutant strain Δ*ypr1* and the *ypr1*-overexpressing strain were constructed previously [15], and the spores of the *T. reesei* Rut-C-30 Δ*ku70* strain and of the corresponding mutants used in this study were stored at –80 °C. Pre-culture was carried out using spores in malt extract agar medium at 28 °C for 7 days [18]; subsequently, fresh conidia were harvested.

For the analysis of vegetative growth, 2 μl of a spore suspension (108 conidia/L) of each strain was inoculated on Mandels–Andreotti (MA) agar plates and incubated at 28 °C [18]. For liquid culture, the inoculum of 10^8 conidia/L was applied to 50 mL of MA medium containing 2% glucose or 2% cellulose, and culture was carried out at 28 °C with shaking at 180 rpm for 72 h. The mycelia that were cultured using glucose were filtered and dried at 70 °C for 48 h, whereas in those that were cultured using cellulose, the intracellular proteins were measured to determine the growth triggered by the insoluble nature of cellulose. To analyze sorbicillinoid production, the supernatant of cultures at the indicated time points was collected at intervals of 24 h and was then used for measuring the absorbance at 370 nm [19].

2.2. Transcriptome analysis via RNA-Seq and KOG-related analysis of DEGs

The mycelia of the *Trichoderma* strains grown for 24 h were harvested by filtration and frozen in liquid nitrogen, and total RNA was isolated using an RNA extraction kit (Sangon Biotech, China) according to the manufacturer's instructions. The quality of the total RNA was examined using an Agilent 2100 Bioanalyzer. The pooled libraries were sequenced on a Novaseq 6000 instrument (Illumina) with read lengths of 300 nt. The number of transcripts per million reads (TPM) was used to determine the transcript expression abundance for genes in all samples. The clean sequence reads obtained were further analyzed by mapping onto the reference genome of *T. reesei* (genome.jgi.doe.gov/Trire2/Trire2.home.html) using the HISAT2 software [20]. The counted transcripts of the transcriptome data were normalized, and statistical analysis was performed using the statistical software package DESeq2 [21]. Data with |log2FC (fold change)| ≥1 and *P*-adjust < 0.05 (adjusted *P* value) were treated as significant DEGs. Both strains were analyzed in two independent biological replicates.

The DEGs obtained from the comparative RNA-Seq analysis were further subjected to a KOG analysis. KOGs that were affected by *ypr1* were calculated as the percentage of genes belonging to a certain KOG in relation to the number of all KOG-assigned genes, based on information from mycocosm.jgi.doe.gov/TrireRUTC-30_1/TrireRUTC-30_1.home.html [17].

2.3. Sequence analysis of the ypr1 protein

A phylogenetic analysis of the Ypr1 protein was conducted via DNAMAN based on the neighbor-joining method. Protein domains were predicted using a CD-search from NCBI (NCBI Conserved Domain Search (nih.gov)).

3. Results and discussion

3.1. Role of ypr1 in fungal growth, conidiation, and secretion of yellow pigments

The Ypr1 protein from *T. reesei* is a type of Zn(II)2Cys6 transcription factor that regulates the synthesis of the sorbicillinoid gene cluster [13]. A protein BLAST analysis showed that Ypr1 orthologs are widely distributed among filamentous fungi, including *Trichoderma, Aspergillus, Penicillium, Neurospora, Pyricularia*, and *Fusarium* species (Fig. 1A, B). In turn, a domain analysis showed that the Ypr1-related proteins contained the fungal transcription factor regulatory middle homology region (Fungal_TF_MHR), in which an N-terminal GAL4-like C6 zinc binuclear cluster DNA-binding domain was located. Gal4 was also among the conserved domains found in most of the orthologs, with the exception of the Ypr1 orthologs from *T. parareesei, P. griseofulvum, A. flavus*, and *A. oryzae* (Fig. 1B).

Growth of the parental *T. reesei* Rut-C-30 Δ*ku70* strain in MA medium led to the secretion of a yellow pigment, which rendered the fermentation broth light yellow; however, the fermentation broth of Δ*ypr1* was white, and the yellow pigment secretion almost disappeared [16], which is consistent with the results reported previously for the wild-type *T. reesei* QM6a [13] and the *T. reseei* QM9414 [15] strains. When the Δ*ypr1* mutant strain was used to detect pigment production under different carbon sources, such as cellulose (inducible carbon source) and glucose (inhibitory carbon source), the pigment-synthesis ability of the *ypr1* deletion mutant strains under the two carbon source conditions could not be observed (Fig. 2A, B). These results indicate that yellow pigment synthesis regulated by Ypr1 is not dependent on inducible or

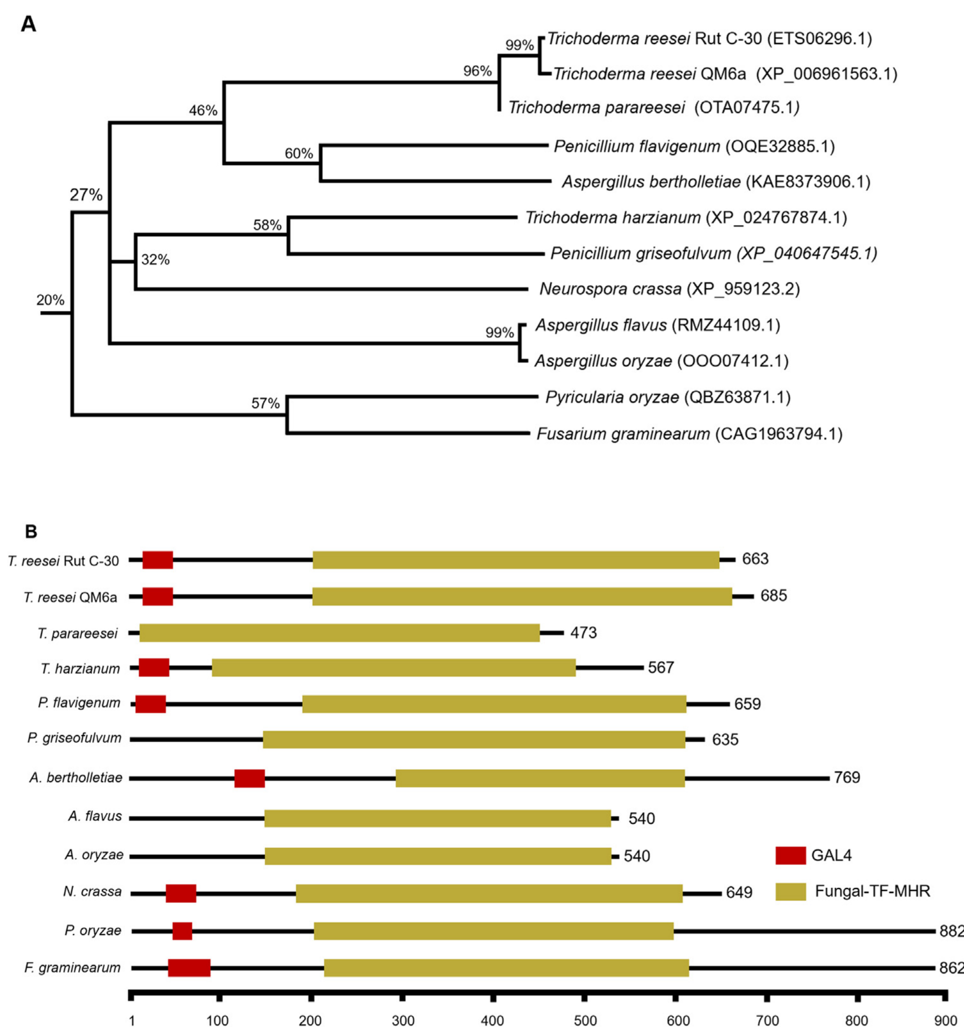

Fig. 1. Phylogenetic analysis and domain prediction of Ypr1 and its orthologs. (A) The phylogenetic analysis of Ypr1 was conducted using DNAMAN based on the neighbor-joining method. (B) Conserved domains of the Ypr1 protein and its orthologs.

inhibitory carbon sources. According to the results of biomass measurements, the mycelial growth rate of the Δ*ypr1* mutant strain tended to be non-significantly higher than that of the parental strain, which may be attributed to the weakening of the secondary metabolic pressure, which eliminates the pigment synthesis caused by the *ypr1*-deficient strain, resulting in a slight promotion of vegetative growth (Fig. 2 C, D).

To verify the effect of *ypr1* overexpression on yellow pigment production in *T. reesei* Rut-C-30, the *ypr1*-overexpressing strain OE*ypr1* was constructed. During the fermentation process, it was observed that the production of yellow pigment from the OE*ypr1* strain was significantly enhanced; in contrast, the vegetative growth of the mutant was impaired, and its mycelial biomass was obviously reduced (Fig. 3). The high yield of yellow pigment led to a significant decrease in the sporulation ability of OE*ypr1* strain [15], which may have been caused by the inhibition of reproductive growth by extracellular sorbicillinoids, or the metabolic burden necessary for excessive pigment production [22]. These results are consistent with the findings obtained using *T. reesei* QM6a and *T. reesei* QM9414 [13,15], suggesting the presence of similar regulatory mechanisms in different *T. reesei* strains.

3.2. Ypr1 modulates the expression of genes involved in both primary and secondary metabolism

To further study the global metabolic regulation of *ypr1* gene knockout on *T. reesei*, the transcriptome was analyzed for the Δ*ypr1* and the control Rut-C-30 Δ*ku70* strain after culture in MA medium using glucose as the sole carbon source at 24 h. The comparative transcriptome analysis showed that there were 722 differentially expressed genes (DEGs) at 24 h in the Δ*ypr1* strain, of which 301 were significantly upregulated and 421 were significantly downregulated ($|\log_2 R| > 1.0$, $P < 0.05$). The significant Pearson's correlation ($R^2 = 0.95$–0.98, $P < 0.001$) and heatmap (Figs S1 and S2) of the TPM read distribution among biological replicates for all samples confirmed the high reproducibility of the sequencing data and samples.

The function of DEGs was categorized according to the Eukaryotic Orthologous Groups (KOG) tool, representing the basic functional groups of genes. KOG classified all genes into four groups, i.e., cellular processes and signaling, information storage and processing, metabolism, and poorly characterized groups, comprising 6885 genes of the total genes. In the current DEG collection, 385 genes were assigned to the KOG groups. To estimate which functional group of genes was most prevalently regulated by Ypr1, the number of overrepresented DEGs in KOG was assessed according to specific rules [17]. After the obtained percentages were compared with the expected percentages for each KOG, higher proportions of DEGs than that expected were allocated into several function collections of "metabolism," including amino acid transport and metabolism, coenzyme transport and metabolism, inorganic ion transport, lipid transport and metabolism, energy production and conversion, and metabolism and secondary metabolite biosynthesis and catabolism. The remaining two classes included defense mechanisms; and posttranslational modification, protein turnover, and chaperones (Fig. S3). In addition, several genes encoding CAZymes, transcrip-

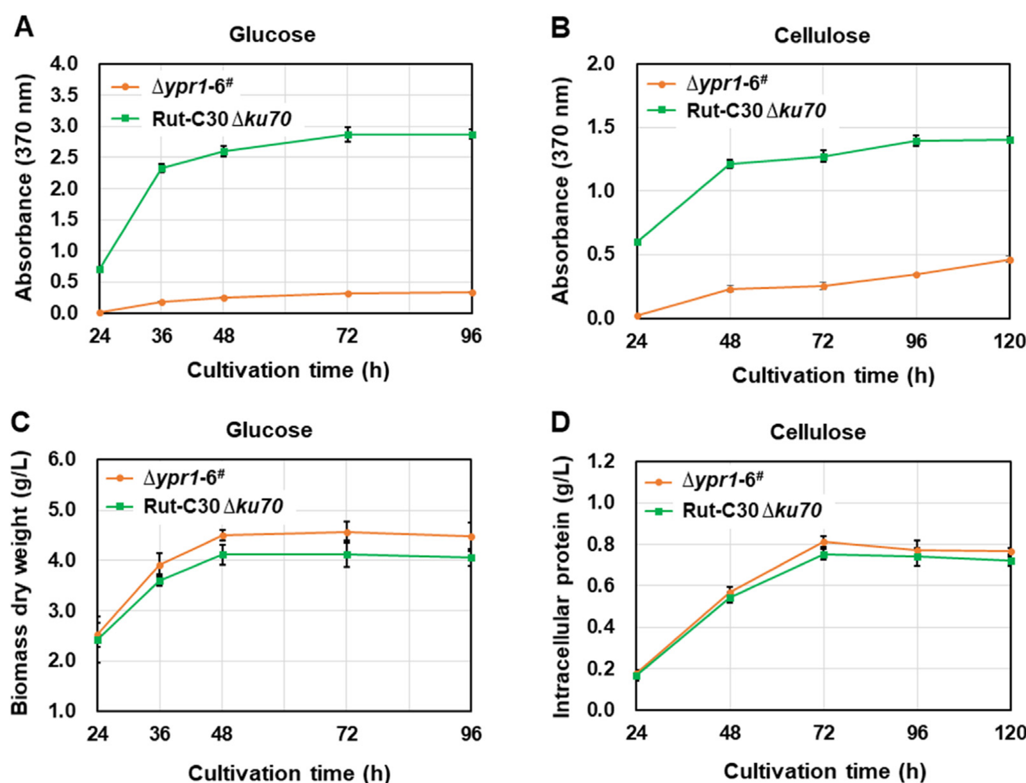

Fig. 2. Yellow pigment production profiles in the culture supernatants of *T. reesei* Rut-C-30 Δ*ku70* and its *ypr1* deletion mutant (Δ*ypr1*–6#), which were cultured using glucose (A) or cellulose (B). Samples were collected at the indicated time point, and the yellow pigment was quantified by measuring the absorbance at 370 nm. The corresponding biomass is presented as the dry weight for glucose culture (C) and intracellular protein for cellulose culture (D).

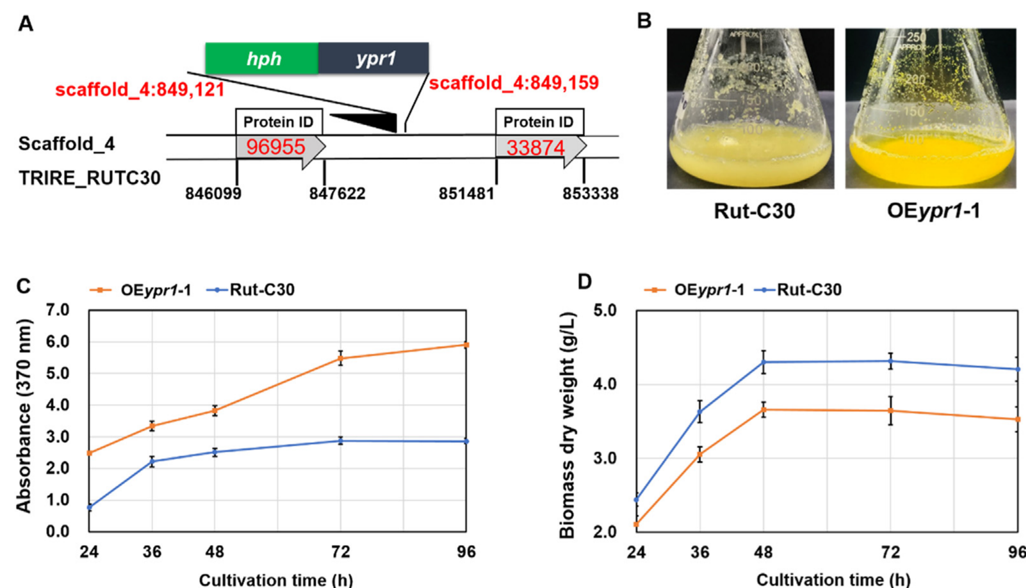

Fig. 3. Effect of *ypr1* overexpression on yellow pigment production. (A) Identification of the insertion site of *ypr1* in the *T. reesei ypr1* overexpression mutant (OE*ypr1*). (B) Image of yellow pigment production in the *T. reesei* strains. (C) and (D) Quantification of the yellow pigment and biomass in the OE*ypr1* mutant and the parent strain, respectively. The strains were cultured using glucose as a carbon source, and samples were collected at the indicated time points.

tion factors, and transporters were also significantly changed in *T. reesei* Δ*ypr1* compared with its parent strain.

The enrichment analysis of the KEGG pathway database for DEGs showed that the DEGs of 10 pathways in the top 20 pathways with enrichment significance were all downregulated significantly by *ypr1* deletion (Fig. 4). Among the large number of downregulated genes with metabolic function, a significant enrichment of genes involved in lipid metabolism (fatty acid synthesis and degradation) was observed. In addition to the acyl-carrier-protein synthetase, the rate-limiting enzyme acetyl-CoA carboxylase was also decreased by over 1-fold, which may explain the negative effect observed on fatty acid biosynthesis. Moreover, the transcript levels of several oxidoreductases and acetyl-CoA acyltransferases in the fatty acid degradation pathway were remarkably decreased.

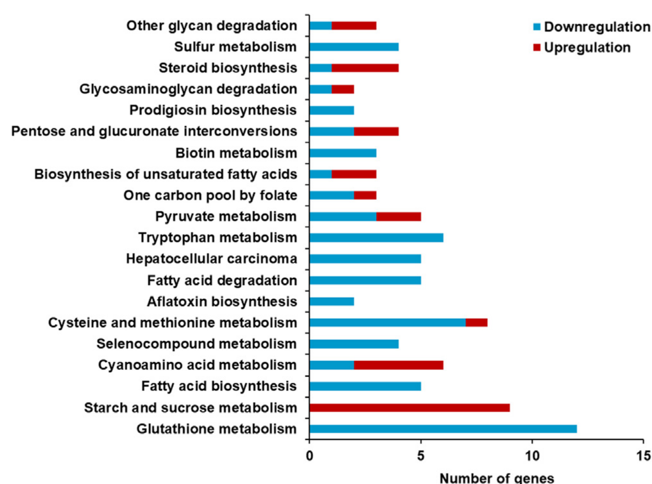

Fig. 4. KEGG enrichment analysis of the DEGs in strain Δ*ypr1* compared with the parental strain Rut-C30 Δ*ku70*. The y-axis indicates the name of the top 20 enriched metabolic pathways.

The glutathione metabolism pathway was weakened. The transcripts of glutathione synthetase (GST), glutamate–cysteine ligase, and dehydrogenases in the glutathione pathway were downregulated, which implies that the reducing power in fungal cells was not sufficiently supported. In particular, the transcripts of genes encoding glutathione *S*-transferases (*TrC-30_35,762* and *TrC-30_10,865*) were significantly downregulated. Moreover, there was a positive correlation between GST activity and aflatoxin production in *Aspergillus spp*., in which GST catalyzes the conjugation of aflatoxin to GSH to excrete aflatoxin derivatives from the mycelium [23]. Whether the downregulated GSH pathway has a negative effect on the secondary metabolism warrants further investigation.

The transcription level of genes related to DNA replication, homologous recombination, and repair was decreased, and the RNA transcription level was weakened because of the downregulation of the transcripts of the basal transcription factors and RNA polymerase II subunits in *T. reesei* Δ*ypr1*. Furthermore, the transcripts of genes encoding spliceosome subunits were decreased by at least 2-fold. The transcriptional decrease observed for genes encoding the ribosomal subunit protein L10e, translation initiation factors (*TrC-30_132,091, TrC-30_23,807* and *TrC-30_92,989*), translation elongation factor (*TrC-30_99,875*), and exosome subunit are detrimental to the assembly of the mature ribosomal 60S subunit for mRNA translation. The transcripts of several genes encoding oxidoreductases and chaperones, which are necessary for protein processing in the endoplasmic reticulum, were significantly downregulated; in turn, the genes encoding proteins in the ubiquitin-mediated proteolysis families also exhibited decreased transcriptional levels. In addition, the transcripts of several genes involved in the amino acid metabolism pathway (including tryptophan, cysteine, histidine, lysine, and methionine metabolism) were downregulated by several fold.

However, some DEGs were upregulated (Fig. 4). The genes that were upregulated in the Δ*ypr1* strain belonged to carbohydrate metabolism pathways, especially genes that regulate starch and sucrose metabolism and the degradation of other polysaccharides. Moreover, genes encoding enzymes involved in glycerophospholipid metabolism, such as methyltrasferase (*TrC-30_130,968*), phosphatidylserine decarboxylase (*TrC-30_132,831*), and fatty-acyl-phospholipid synthase (*TrC-30_137,899*), were also significantly upregulated in *T. reesei* Δ*ypr1*. Furthermore, the *TrC-30_25,484* gene, which encodes the squalene synthetase that participates in steroid or terpenoid synthesis, was upregulated by 1-fold in *T. reesei* Δ*ypr1*, and the genes encoding cytochrome P450 (*TrC-30_107,898*) and sterol reductase (*TrC-30_72,950*), which are engaged in zymosterol the synthesis pathway, were upregulated by over 1-fold, suggesting that the secondary metabolism is regulated by Ypr1.

Overall, the deletion of Ypr1 had remarkable effects on the metabolism of *T. reesei* Rut-C-30 Δ*ku70* (Table S1). With the exception of the extracellular glycoside hydrolases and steroid and terpenoid biosynthesis, a large number of DEGs encoding proteins that participate in DNA replication; mRNA and protein processing; amino acid, coenzyme, and energy metabolism in the peroxisome; and fatty acid biosynthesis/degradation exhibited decreased transcript levels, which indicated that the primary metabolism in *T. reesei* Δ*ypr1* was inactivated. Furthermore, about 65% of the DEGs involved in secondary metabolite catabolism were also downregulated, which is consistent with the yellow pigment production from the Δ*ypr1* strain. Because of the cessation of pigment production, the Δ*ypr1* strain exhibited reduced consumption of coenzymes, energy, and other cofactors. However, some DEGs involved in secondary metabolism were upregulated, as were those in the amino acid and lipid metabolism groups, indicating that other primary or secondary metabolism remained active.

3.3. Ypr1 regulates the expression of the *pks* and *nrps* genes

According to the results of the transcriptomics analysis, the difference between the percentage of obtained DEGs (13.25%) and the expected percentage (3.82%) in secondary metabolism classes was remarkable. Genes encoding short-chain dehydrogenase/reductases, cytochrome p450 monooxygenases, and flavin-dependent monooxygenases related to secondary metabolism were downregulated in *T. reesei* Δ*ypr1,* which adversely affected the expression of specific clusters of secondary metabolites in this strain. As expected, the transcripts of whole PKS_11 cluster genes encoding sorbicillinoid precursors were absent, including two polyketide synthases (*TrC-30_93,844* and *TrC-30_93,847*), the regulator Ypr2 (*TrC-30_31,634*), and an auxiliary modifier (Fig. 6), which resulted in the disappearance of pigment secretion [13].

In addition to the downregulation of the PKS_11 cluster, which is regulated by Ypr1 and in which the position effect of the regulator may play a crucial role, other secondary metabolite clusters were also affected by Ypr1 at different levels (Fig. 5, Table 1). Obviously, the deletion of *ypr1* significantly activated the whole PKS_2 gene cluster, including the main polyketide synthase, methyltransferase, β-lactamase, and corresponding auxiliary modifiers. The level of the NRPS_21 (*TrC-30_77,486*) transcript, encoding a non-ribosomal peptide synthetase, was downregulated by about two-fold in *T. reesei* Δ*ypr1*; moreover, this transcript is an ortholog of SirP and synthesizes the epipolythiodioxopiperazine phytotoxin sirodesmin PL in *Leptosphaeria maculans* [24]. In the PKS_2 cluster, the level of the transcript of the polyketide synthase gene (*TrC-30_90,904*) was improved by 64-fold in *T. reesei* Δ*ypr1*, in which the homologs *adaA* from *Aspergillus niger* [25] and *vrtA* from *Penicillium aethiopicum* [26] are involved in anthracenone and naphthacenedione biosynthesis, respectively. Another two non-ribosomal peptide synthetase gene clusters, NRPS_28 including the *TrC-30_38,585* and *TrC-30_142,783* genes, were all activated by over one-fold in *T. reesei* Δ*ypr1*, in which *TrC-30_142,783* encodes a 14-module peptaibol synthetase [27].

The secondary metabolism is mediated by substrates (such as acyl-CoA, amino acids, nucleotides, and carbohydrates), cofactors, metabolites, and the energy produced during primary metabolism. Moreover, the secondary metabolism always shares the same enzymes with the primary metabolism, such as oxidoreductases, monooxygenases, dehydrogenases, and acyl (malonyl)-CoA transferases. In fact, the perturbation of fatty acid metabolism can enhance the yield of various secondary metabolites because of the sharing of precursor molecules between the two pathways, such as acetyl-CoA and malonyl-CoA [28]. Therefore, in *T. reesei* Δ*ypr1*, the downregulation of acetyl-CoA carboxylase, acyl-carrier-protein synthetase, and short-chain dehydrogenases in the fatty acid biosynthesis pathway is beneficial for the accumulation of acyl-CoA, as precursors of secondary metabolites.

In the course of the secondary biosynthetic and export pathway, transporters, especially those in the major facilitator superfamily (MFS),

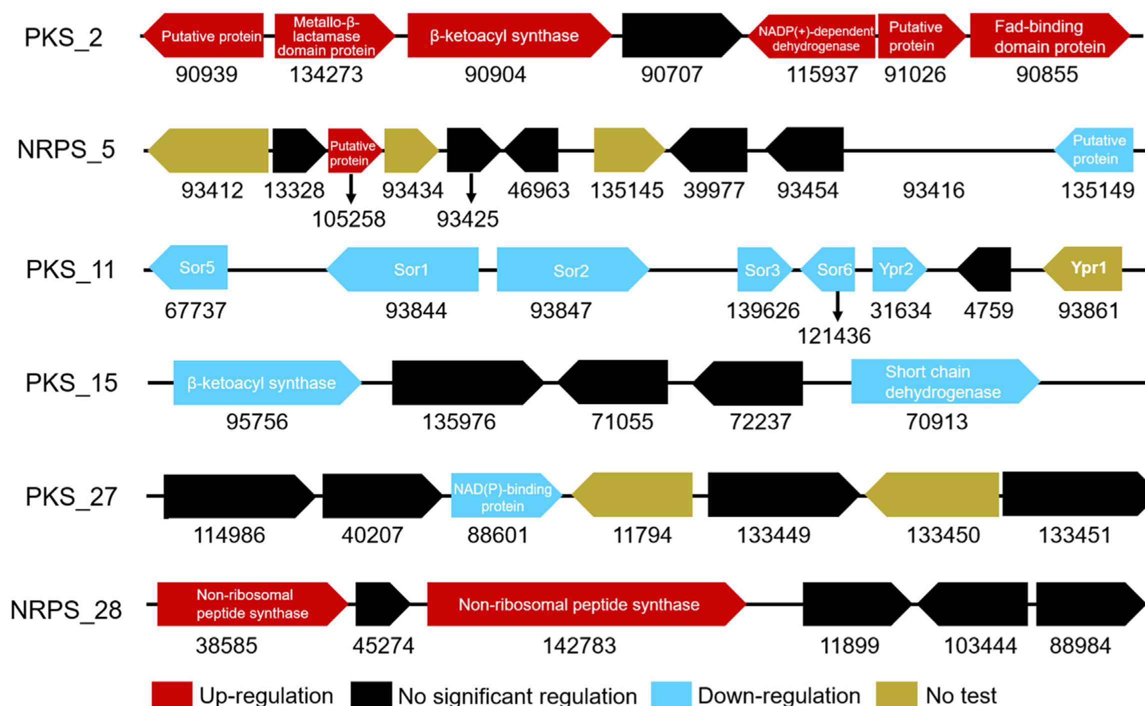

Fig. 5. Transcriptional changes of genes located in the selected secondary metabolite synthetic gene clusters in *T. reesei* Δ*ypr1* compared with the parental strain. The red, black, blue, and golden colors indicate upregulation, no significant change, downregulation, and no detectable transcription levels, respectively.

always play a crucial rule [29]. The comparison of *T. reesei* Δ*ypr1* with the parent strain identified 40 DEGs, including five genes encoding sugar transporters that were classified in the KOG transporters sub-class. In addition, the transcripts of genes encoding several MFS transporters of cations and ammonium were also remarkably changed, whereas the number of up- and down- regulated DEGs encoding transporters in *T. reesei* Δ*ypr1* was kept at a similar level, and its locus is located far from the annotated secondary metabolite clusters. Nevertheless, their functional roles in the metabolism pathway remain unclear.

Furthermore, molecules in the signal transduction pathways, such as heterotrimeric G-proteins, protein kinase A (PKA), MAP kinase, and cAMP, and global regulators play an important role in the secondary metabolite pathways [30]. Many DEGs encoding protein kinases and related G-proteins were found in *T. reesei* Δ*ypr1*; however, their functions in secondary metabolite pathways warrant further characterization. The Xpp1 transcription factor of *T. reesei* acts as a switch between the primary and secondary metabolism [17]. This also indicates the enhancement of the expression of the regulator *Ypr1* and *PKS* genes, which encode yellow pigments (*TrC-30_93,844* and *TrC-30_93,847*), and the downregulation of *TrC-30_90,904* transcripts, which are regulated by *Ypr1* and other *PKS* genes, such as *TrC-30_129,024* and *TrC-30_37,393*, in the *T. reesei* Δ*xpp1* strain [17]. Interestingly, the DEGs from the Δ*ypr1* strain did not include the *xpp1* gene, which implies that the *ypr1* gene

Fig. 6. Genomic organization of the SOR cluster and its neighboring cellulase-related genes in *T. reesei*, and transcription levels of these genes in strain Δ*ypr1* relative to Rut-C-30 Δ*ku70*. Inf: the transcript of *ypr1* was not detected in Δ*ypr1*, but as present in Rut-C-30 Δ*ku70*.

Table 1
Effect of ypr1 deletion on the expression of secondary metabolites in T. reesei*.

Protein ID	Description	Mean reads	Log_2 (fold change)	Adjusted P value
93,844	beta-ketoacyl synthase	256	−7.93	3.22E-209
93,847	beta-ketoacyl synthase	36.5	−10.58	1.87E-295
95,756	beta-ketoacyl synthase	182.5	−1.28	0.00332392
38,585	non-ribosomal peptide synthase	1808.9	1.09	0.000740733
142,783	non-ribosomal peptide synthase	1128	1.09	0.001927682
90,904	beta-ketoacyl synthase	2011.5	5.99	1.19E-73
77,486	non-ribosomal peptide synthase	43	−1.43	0.01870513

* The data shown are the differential transcription levels between the deletion mutant and the wild-type strain *T. reesei* Rut-C30 Δ*ku70*.

Table 2
Main DEGs related to (hemi)cellulase in the Δ*ypr1* strain.

Gene ID[a]	Gene name	Description	Log_2R[b]
125,125	*cbh1*	Exoglucanase 1	1.00
122,470	*cbh2*	Exoglucanase 2	1.78
5304	*egl1*	Endoglucanase I precursor	2.27
72,489	*egl2*	Endoglucanase II	1.69
124,438	*egl3*	Endoglucanase III	1.71
25,940	*egl5*	Endo-1,4-β-glucanase V	1.06
136,547	*bgl1*	β-D-glucoside glucohydrolase I	1.52
74,305	*cel3e*	β-glucosidase	1.31
127,115	*cel1a*	β-glucosidase	1.50
77,989	*cel1b*	β-glucosidase	1.20
38,418	*xyn1*	Endo-1,4-β-xylanase 1	1.89
23,616	*xyn3*	Xylanase III	1.38
90,847	*xyn4*	Endo-1,4-β-xylanase 4	1.23
134,945	*xyn5*	Endo-1,4-β-xylanase 5	1.31
140,746	*bxl1*	GH3 family β-xylosidase	1.10
104,220	*swo1*	Swollenin	1.42
121,449	*cip1*	Hypothetical protein	1.40
98,788	*xyr1*	Xylanase regulator 1	1.58
98,455	*ace3*	Activator of cellulase expression	1.18
109,243	*crt1*	General substrate transporter	1.62
9778	*lae1*	Methyl transferase	1.08

[a] Gene ID was assigned based on the *T. reesei* RUT-C-30 genome database (https://www.ncbi.nlm.nih.gov/genome/323?genome_assembly_id=49,799).
[b] R: Ratio of the transcription of genes in *T. reesei* Δ*ypr1* over that in *T. reesei* Rut-C-30 Δ*ku70*. Genes were differentially expressed between the two strains when the average reads of the corresponding transcripts differed with |log2FC| ≥ 1 and adjusted $P < 0.05$.

may be a downstream gene of *xpp1*. Moreover, the expression of the VELVET protein complex, which includes the Lae1, Vel1, and VelB methyltransferases, in several fungal strains is crucial for secondary metabolite catabolism and development [31–33]. Although the deletion of Lae1 only had an effect on five secondary metabolite clusters in *T. reesei*, ceased yellow pigment production, and reduced sporulation, Lae1 regulates different secondary metabolite clusters compared with Ypr1 [34]. Interestingly, the transcription of *lae1* was improved by 1-fold in the Δ*ypr1* strain. However, the expression of the corresponding secondary clusters regulated by Lae1 did not change after *ypr1* deletion.

3.4. Regulation of the crosstalk of ypr1 between cellulase and yellow pigment production

In *T. reesei* Δ*ypr1*, the upregulation of the crucial activators of cellulase production, i.e., Xyr1 (3-fold), Ace3 (2.23-fold), and positive methyltransferase Lae1 (2.1-fold) [35], was accompanied by a significant increase in the abundance of the transcripts of genes encoding extracellular glycoside hydrolases, especially major cellulases and hemicellulases; moreover, the transcripts of the cellobiohydrolase genes *cbh1* (*TrC-30_125,125*) and *cbh2* (*TrC-30_122,470*); and of the endoglucanase genes *eg1* (*TrC-30_5304*), *eg2* (*TrC-30_72,489*), and β-glucosidase *bgl* (*TrC-30_136,547*) were increased by 2-, 3.4-, 4.8-, 3.2-, and 2.9-fold, respectively, whereas the transcripts of the major xylanase genes *xyn1* (*TrC-30_38,418*), *xyn4* (*TrC-30_90,847*), *xyn5* (*TrC-30_134,945*), and β-xylosidase *bxl1* (*TrC-30_140,746*) were increased by 3.7-, 2.3-, 2.5-, and 2.1-fold (Table 2).

Interestingly, four cellulase-production-related genes adjacent to the PKS gene cluster, including *TrC-30_69,551, axe1* (encoding an acetylxylanesterase), *cip1* (encoding a cellulase-inducible protein), and *cel61a* (*egl4*) (encoding a GH61-family accessory protein), form a “sorbicillinoid–cellulase” super cluster [36] (Fig. 6), indicating that the secondary metabolites may be coupled with cellulase production. This super cluster was also detected in other species, such as *Fusarium fujikuroi* [37]. In contrast to the downregulated SOR cluster genes, with the exception of *cel61a*, the transcription levels of these genes were significantly upregulated in Δ*ypr1*, indicating that the deletion of Ypr1 facilitated the transcription of adjacent supercluster genes. How this “sorbicillinoid–cellulase” super cluster affects yellow pigment and cellulase production is worthy of future study.

4. Conclusion

The global changes in the transcription profile of the industrial cellulase producer *T. reesei* Rut-C-30 after *ypr1* deletion were reported here. A large number of DEGs encoding proteins involved in DNA replication, mRNA, protein processing, amino acids, coenzymes, and energy metabolism, as well as fatty acid biosynthesis/degradation, exhibited decreased transcript levels after *ypr1* deletion, suggesting that Ypr1 is critical for both primary metabolism and secondary metabolism. In addition to regulating yellow pigment production, Ypr1 also regulates the PKS_2 and NRPS_28 gene clusters, as well as the “sorbicillinoid–cellulase” super cluster. The results of this study provide a basis for further engineering of *T. reesei* to enhance the production of bioactive agents.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.engmic.2022.100065.

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