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Research Article

Protein Expression Profile and Transcriptome Characterization of *Penicillium expansum* Induced by *Meyerozyma guilliermondii*

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Antagonistic yeasts can inhibit fungal growth. In our previous research, *Meyerozyma guilliermondii*, one of the antagonistic yeasts, exhibited antagonistic activity against *Penicillium expansum*. However, the mechanisms, especially the molecular mechanisms of inhibiting activity of *M. guilliermondii*, are not clear. In this study, the protein expression profile and transcriptome characterization of *P. expansum* induced by *M. guilliermondii* were investigated. In *P. expansum* induced by *M. guilliermondii*, 66 proteins were identified as differentially expressed, among them six proteins were upregulated and 60 proteins were downregulated, which were associated with oxidative phosphorylation, ATP synthesis, basal metabolism, and response regulation. Simultaneously, a transcriptomic approach based on RNA-Seq was applied to annotate the genome of *P. expansum* and then studied the changes of gene expression in *P. expansum* treated with *M. guilliermondii*. The results showed that differentially expressed genes such as *HEAT*, *Phosphoesterase*, *Polyketide synthase, ATPase*, and *Ras-association* were significantly downregulated, in contrast to *Cytochromes P450*, *Phosphatidate cytidylyltransferase*, and *Glutathione S-transferase*, which were significantly upregulated. Interestingly, the downregulated differentially expressed proteins and genes have a corresponding relationship; these results revealed that these proteins and genes were important in the growth of *P. expansum* treated with *M. guilliermondii*.

1. Introduction

Penicillium expansum is one of the most common pathogens in pears which cause blue mold decay and is able to secrete toxic secondary metabolite patulin (PAT), causing serious food safety problems and harming human health [1]. Recently, there are many strategies that have been employed to control the postharvest diseases of pears; among them chemical and physical methods are contribute the major part. However, both methods have some drawbacks. Therefore, safe and efficient antagonistic yeasts have been a research hotspot in the control of postharvest diseases of pear. *Cryptococcus laurentii*, *Rhodotorula glutinis*, *Rhodosporidium paludigenum*, and *Rhodotorula mucilaginosa* [2–5] are some effective biocontrol agents against *P. expansum* in pears.

Meyerozyma guilliermondii was reported as an effective antagonistic yeast, which significantly controlled blue mold decay of pears [6] and controlled rice blast disease, cabbage black leaf spot disorder, and bacterial wilt caused by *Ralstonia solanacearum*, a tomato pathogen [7]. It also showed significant biocontrol of gray mold disease on table grapes caused by *Botrytis cinerea* [8] and reduced the severity of rot in mangoes during storage [9]. Our previous study showed that blue mold decay caused by *P. expansum* was significantly inhibited by *M. guilliermondii* without any change in the fruit quality [6]. The biocontrol efficacy increased with increasing yeast concentrations. *M. guilliermondii* colonized in pears rapidly and maintained relatively higher numbers to compete for nutrient and space with pathogen. *M. guilliermondii* also enhanced the defense to pathogens in pears [6]. The proteomics and transcriptomics analysis conducted in pears induced by *M. guilliermondii* revealed that *M. guilliermondii* could upregulate the expression of defense-related proteins and genes of pears [6, 10].

Several proteins intervene during the interaction of pathogens with antagonistic yeasts and many of them are crucial to explain the inhibition mechanism of antagonistic yeast. Our previous research reported that more than one-third of the proteins differentially expressed in *Talaromyces rugulosus* in response to *Yarrowia lipolytica* were associated with essential metabolism, like phosphoglycerate kinase, nucleoside diphosphate kinase, and so on, which showed that the mechanisms by which *Y. lipolytica* inhibited *T. rugulosus* involved in the essential metabolism [11]. In the same manner, a general analysis of transcriptome and proteome modification of *P. expansum* spores during germination was conducted by Zhou et al. using RNA-Seq and iTRAQ approaches. The corresponding result showed a statistic of 3026 genes and 489 proteins which were differentially expressed [12]. However, as far as we know, the molecular mechanism of *M. guilliermondii* against *P. expansum* has not been studied yet. In the present work, we explored the differentially expressed proteins and several defense-related genes of *P. expansum* cocultured with *M. guilliermondii* through proteomics and transcriptomics analysis and tried to establish the molecular mechanism of *M. guilliermondii* inhibiting the growth of *P. expansum*.

2. Materials and Methods

2.1. Yeast. The antagonist yeast *M. guilliermondii* (preserved in the China Center for Type Culture Collection, No. M2017270) was isolated from unsprayed orchards. The yeast was cultured in nutrient yeast dextrose broth medium (NYDB, nutrient broth 8 g/L, yeast extract 5 g/L, and glucose 10 g/L) on an incubator shaker (180 rpm, 28°C) for 20 h. After incubation, yeast cells were collected by centrifugation (6918 ×g for 10 min); the pellets were resuspended in sterile water and adjusted to 1×10^8 cells/mL concentration with a hemocytometer.

2.2. Pathogen. P. expansum was maintained on potato dextrose agar medium at 4°C. Before using, the *P. expansum* strain was inoculated in PDA plates and allowed to grow for seven days at 25°C in an incubator. After seven days, the spores were removed from the Petri dish and suspended in sterile distilled water. A hemocytometer was used to adjust spore concentrations to 1×10^7 spores/mL.

2.3. Analysis of the Differentially Expressed Proteins of P. expansum and P. expansum Incubated with M. guilliermondii. Initially, 1 mL of spore suspension (1×10^7 spores/mL) of *P. expansum* was added to 100 mL of PDB inside 500 mL Erlenmeyer volumetric flasks and incubated for two days at 25°C, 120 rpm. Then, 1 mL suspension of *M. guilliermondii* at 1×10^8 cells/mL was added. After 1 d incubation, the mycelia were collected and the protein was extracted according to the method described by Yang et al. [11].

2-DE and Image Analysis were conducted in accordance with the method delineated by Yang et al. and Zhang et al. with some modifications [11, 13, 14]. Isoelectric focusing was used to separate proteins in GE Ettan IPGphor 3, according to the manufacturer's instructions [14].

Identification of Proteins. Mass calibrations were carried out with a standard peptide mixture. Mass spectra were acquired using Matrix-Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF) mass spectrometer (Bruker Daltonics, Germany). The identification of the vast majority of proteins was performed using search engine MASCOT Peptide Mass Fingerprint of Matrix Science and compared with NCBInr and Swiss-Prot databases. The parameters used for MS search were taxonomy, all series; allowed modifications, carbamidomethyl of cysteine (fixed), oxidation of methionine (variable); and peptide tolerance, ±0.3 Da. Only the highest Mowse score was considered as the most probable identification and was significant ($P < 0.05$) when protein scores were greater than 88 (NCBInr) or 70 (Swiss-Prot) [14].

2.4. Transcriptomic Analysis of P. expansum and P. expansum Incubated with M. guilliermondii. RNA from the mycelia was extracted in accordance with the prescription of Sangon Co., Shanghai, China, with little modifications [14]. The concentration of RNA was detected by Qubit® RNA Assay Kit in Qubit® 2.0 Fluorometer (Life Technologies, Carlsbad, CA, USA). Nanodrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA, USA) was used to determine the purity of RNA. RNA Nano 6000 Assay Kit of the Bioanalyzer 2100 system was used (Agilent Technologies, Santa Clara, CA, USA) to assess the integrity of the RNA. The samples that met the experimental requirements were used for the next steps [14].

2.4.1. Transcriptome Analysis. Each RNA sample was used to perform RNA-Seq. tRNA-Seq libraries were sequenced on an Illumina HiSeq 2500 platform to generate 125 bp/150 bp single-ended reads [14].

2.4.2. Bioinformatics Analysis of RNA-Seq Data. Transcriptomic data were assembled, after high-quality sequencing data were acquired, using Trinity [14]. The UniGene sequences of *P. expansum* and *P. expansum* incubated with *M. guilliermondii* were searched using BLAST and compared with those obtained using the NR, Swiss-Prot, GO, and KEGG databases for the confirmation of amino acid sequence.

2.5. Validation of RNA-Seq Data by RT-qPCR. RT-qPCR analysis was performed using RNA extracted from *P. expansum* and *P. expansum* incubated with *M. guilliermondii*, in order to validate the data obtained from RNA-Seq. The genes and their specific primers used for RT-qPCR were listed in Supplementary Table 1 and analysis was performed using a Bio-Rad CFX-96 Real-Time PCR System (Bio-Rad, USA). The reaction system that was conducted in accordance with the method delineated by Yang et al. [14] comprised of 12.5 μL SYBR® Premix Ex Taq™ II (2x); 0.5 μL

50x Rox Reference Dye II; 1 μL Primer-F; 1 μL Primer-R; 2 μL cDNA; and 8 μL ddH_2O. The thermocycler conditions were set at an initial denaturation temperature of 95°C for 30 s, followed by 40 cycles of 95°C for 5 s and finally, one cycle of 60°C for 34 s and 72°C for 30 s. To normalize the gene expression level, Actin gene from *P. expansum* was used as an internal control. The RT-qPCR analysis was repeated three times with three technical replicates. The relative expression level of the sample gene was calculated using a $2^{-\Delta\Delta CT}$ method [10].

2.6. Statistical Analysis. The data were analyzed by analysis of variance (ANOVA) using the statistical program SPSS/PC version 8 (SPSS Inc., Chicago, Illinois, USA), and Duncan's multiple range test was used for mean separation. The statistical significance was assessed at $P < 0.05$.

3. Results

3.1. Identification of Differentially Expressed Proteins of P. expansum. The whole protein expression of *P. expansum* and *P. expansum* treated with by *M. guilliermondii* was shown in Figure 1. In each gel, a total of 66 differentially expressed (average fold change ≥ 2, $p < 0.05$) proteins were identified. Just 6 of them were significantly upregulated, while 60 proteins were significantly downregulated. Furthermore, 43 spots which showed the best resolution among the significantly differentially expressed proteins were analyzed for identification by mass spectrometry (MS). An elaborated report on lowercase letters about the names of peptides is in Table 1.

The basic information of 43 differentially expressed protein spots, comprising isoelectric point, molecular weight, and peptide matches, was assigned to a class. As reported in Table 1, several proteins were related to secondary metabolite synthesis, which included polyketide synthase (spot 20), enoylreductase (spot 32). Some proteins were associated with ATP synthesis, which included ATP hydrolase (ATPase), delta/epsilon subunit F1, N-terminal (spot 17), ATPase, F1 complex beta subunit/V1 complex, and C-terminal (spot 38). Some proteins were also associated with cellular basal metabolism, which included phosphoesterase (spot 36), glyceraldehyde/erythrose phosphate dehydrogenase family (spot 44), and phosphoglycerate kinase (spot 41) and some proteins were found to be associated with environmental immune response, which included heat shock 70 kDa protein (spot 24); these downregulated proteins were all associated with the basal metabolic process, response, and regulation of *P. expansum*. Gene ontology (GO) functional annotation examination was performed for the whole identified proteins, which exposed a broad number of molecular functions like biological processes and cellular components (Figure 2). The results indicated that the largest group of biological processes was metabolic process (25 proteins) and cellular process (25 proteins), and the other two biological processes were single-organism process (16 proteins) and biological regulation (7 proteins); these proteins were all related to the basal metabolic process. All identified proteins were annotated to categories. There were 98 and 90 proteins involved in cellular and biological processes as well as 56 proteins involved in molecular functioning, respectively. All the differentially expressed proteins were mainly involved in basic metabolism (30%), binding (23%), catalytic (21%), transporter (7%), and hypothetical (7%) processes (Figure 3).

3.2. Transcriptomic Analysis by RNA-Seq. The transcriptomes of *P. expansum* and *P. expansum* cocultured with *M. guilliermondii* were analyzed using RNA-Seq technology. The transcriptome data indicated a total number of 13 Gb clean data; the Q30 base percentage was 89.75% and 89.92% (Table 2). A total of 434 differentially expressed genes (DEGs) were compiled, among them 408 genes were upregulated and 26 genes were downregulated in the *P. expansum* treated with *M. guilliermondii* ($|\log_2$ (fold change)$| \geq 2$, FDR < 0.05) (Table 3). Using gene ontology (GO), these DEGs were clustered by gene function (Figure 4). The cellular components and molecular functions were further analyzed, each contained 19 subgroups and the biological processes contained 22 subgroups. The main categories concerning cellular component contained cell (23.9%), cell part (23.9%), organelle (13.9%), and membrane (13.4%). The highest percentage of identified differentially expressed genes under molecular function category included catalytic activity (46.1%) and binding (38.5%), whereas the percentage in the biological process category was as follows: basic metabolism (21.0%), cellular process (20.2%), single-organism process (19.0%), and biological regulation (18.5%). The most enriched KEGG pathway of *P. expansum* transcriptome analysis was shown in Figure 5. There were four pathways including cellular processes, environmental information processing, genetic information processing, and metabolism. In the cellular processes, there were 5 DEGs in cell growth and death, 16 DEGs in transport and catabolism. In the environmental information processing, there were 22 DEGs in folding, sorting, and degradation, 6 DEGs in transcription, and 6 DEGs in translation. In metabolism, the highest pathway is carbohydrate metabolism (32.14%), energy metabolism (17.26%), amino acid metabolism (16.07%), and lipid metabolism (14.28%).

3.3. Validation of RNA-Seq Data by RT-qPCR. The genes identified as key DEGs of *P. expansum*, due to *M. guilliermondii* coculturing, which are involved in basal metabolism were evaluated by real-time-quantitative polymerase chain reaction (RT-qPCR) (Table 4, Figure 6). From the result of RNA-Seq (Figure 6), the expression levels of TRINITY_DN7572_c0_g2 (*Phosphoesterase*), TRINITY_DN10607_c0_g1 (*Polyketide synthase, enoylreductase*), TRINITY_DN3524_c0_g2 (*ATPase, F0/V0 complex, subunit C*), TRINITY_DN7527_c0_g1 (*ATPase, F1/A1 complex, alpha subunit, N-terminal*), TRINITY_DN5224_c0_g4 (*HEAT, type 2*) were significantly different. Expression levels of these five genes were significantly decreased, which were 0.53, 0.21, 0.44, 0.41, and 0.32 times lower than those in control group, respectively. The results of RT-qPCR were consistent with the gene expression results of RNA-Seq.

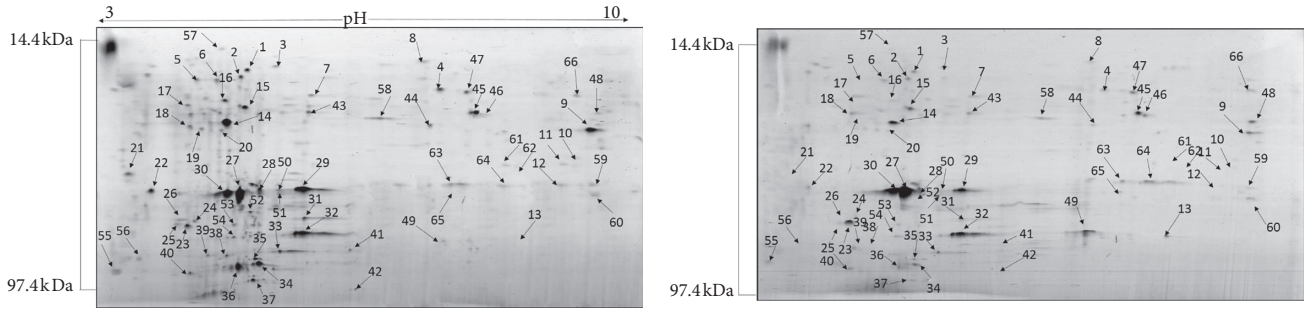

Figure 1: 2-D gels of differentially expressed proteins of *P. expansum* treated with sterile distilled water and *M. guilliermondii*. (a) *P. expansum* protein expressed after treatment with sterile distilled water. (b) *P. expansum* protein expressed after treatment with *M. guilliermondii*.

Table 1: Identification of differentially expressed proteins in *P. expansum* after different treatments.

Protein spot	Protein name	NCBI accession	Mass	PI	Species	Score
↓1	Hypothetical protein PDIG_00280	gi|425781927	10361	5.28	*Penicillium digitatum*	397
↓2	Thioredoxin	gi|700488450	11987	5.12	*Penicillium expansum*	79
↓4	40S ribosomal protein S21	gi|584409470	10008	7.77	*Penicillium roqueforti*	162
↓6	Profiling	gi|768690728	13945	4.63	*Penicillium solitum*	96
↓7	Pc21g11730	gi|255954903	13188	5.59	*Penicillium rubens Wisconsin*	148
↓8	UBI 3 fusion protein (149 AA)	gi|3086	18932	9.82	*Neurospora crassa*	86
↓9	Cyclophilin-type peptidyl-prolyl cis-trans isomerase	gi|700447277	28161	8.91	*Penicillium expansum*	222
↓14	Redoxin	gi|700458248	18736	8.69	*Penicillium expansum*	115
↓15	Pc06g01290	gi|255930353	14246	5.39	*Penicillium rubens Wisconsin*	166
↓16	Aegerolysin	gi|700452244	15646	4.92	*Penicillium expansum*	124
↓17	ATPase, F1 complex, delta/epsilon subunit, N-terminal	gi|700448219	17639	5.19	*Penicillium expansum*	171
↓20	Polyketide synthase, enoylreductase	gi|700457792	37941	5.6	*Penicillium expansum*	116
↓22	Hypothetical protein BC1G_05980	gi|154311893	22792	4.29	*Botrytis cinerea*	98
↑23	Peptidase aspartic, catalytic	gi|700446168	43688	5.57	*Penicillium expansum*	669
↓24	Heat shock 70 kDa protein	gi|425774732	69862	5.03	*Penicillium digitatum*	271
↓25	Elongation factor 1 beta central acidic region, eukaryote	gi|700446479	25224	4.47	*Penicillium expansum*	159
↓26	Nascent polypeptide-associated complex (NAC) subunit, putative	gi|425773866	21921	4.75	*Penicillium digitatum*	156
↓28	Pc22g10220	gi|255948526	67030	5.32	*Penicillium rubens Wisconsin*	221
↓29	Glucose/ribitol dehydrogenase	gi|700452783	28635	6.97	*Penicillium expansum*	240
↓31	Ketose-bisphosphate aldolase, class-II	gi|700454353	39308	4.91	*Penicillium expansum*	390
↓32	Polyketide synthase, enoylreductase	gi|700457792	37941	5.6	*Penicillium expansum*	316
↓33	Enolase BAC82549-Penicillium chrysogenum	gi|255938796	47250	5.26	*Penicillium rubens Wisconsin*	313
↓34	Chaperonin Cpn60	gi|700454561	61890	5.61	*Penicillium expansum*	368
↓35	Glutamate carboxypeptidase, putative	gi|425778622	53077	5.34	*Penicillium digitatum*	450
↓36	Phosphoesterase	gi|700450431	50668	5.18	*Penicillium expansum*	166
↓37	Chaperone DnaK	gi|700451345	72353	5.51	*Penicillium expansum*	356
↓38	ATPase, F1 complex beta subunit/V1 complex, C-terminal	gi|700446699	55307	5.34	*Penicillium expansum*	535
↓39	Hypothetical protein PDIG_75600	gi|425768525	61928	4.99	*Penicillium digitatum*	135
↓40	Protein disulfide isomerase	gi|700453960	56761	4.66	*Penicillium expansum*	112
↓41	Phosphoglycerate kinase	gi|417486	44103	6.07	*Penicillium citrinum*	96
↓43	Lithostathine precursor	gi|45430003	19720	5.75	*Bos taurus*	222
↓44	Glyceraldehyde/erythrose phosphate dehydrogenase family	gi|700447332	36225	6.01	*Penicillium expansum*	126
↓45	Nucleoside diphosphate kinase	gi|425772472	16737	7.77	*Penicillium digitatum*	259
↓47	FK506-binding protein	gi|584415065	13203	6.41	*Penicillium roqueforti*	328

Table 1: Continued.

Protein spot	Protein name	NCBI accession	Mass	PI	Species	Score
↑48	Pc16g13060	gi|255941672	18109	6.91	*Penicillium rubens Wisconsin*	256
↑49	Pc21g22820	gi|255956993	37501	8.07	*Penicillium rubens Wisconsin*	215
↓51	Thiamine pyrophosphate enzyme, C-terminal TPP-binding	gi|700446530	63440	5.69	*Penicillium expansum*	185
↓52	Proteasome subunit alpha type 3	gi|70991357	29766	5.19	*Aspergillus fumigatus*	82
↓53	2-Phosphoglycerate dehydratase	gi|74662366	47264	5.14	*Penicillium chrysogenum*	246
↓54	Flagellin	gi|736787790	38621	5.09	*Ewingella americana*	229
↓60	Pc22g17950	gi|255949874	36031	9.47	*Penicillium rubens Wisconsin*	75
↓61	Nucleic acid-binding, OB-fold	gi|700454689	58601	6.54	*Penicillium expansum*	70
↓62	Phosphoglycerate kinase	gi|417486	44103	6.07	*Penicillium citrinum*	177

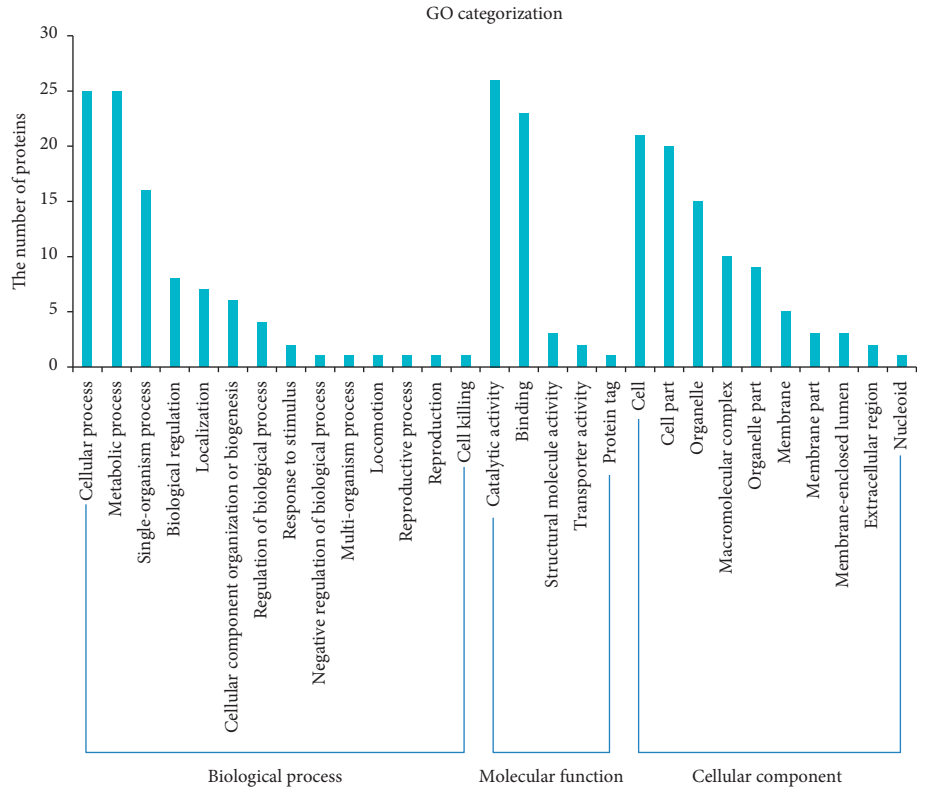

Figure 2: GO categorization of differentially expressed proteins in *P. expansum* after different treatments. The proteins were categorized according to the annotation of GO, and the number of each category is displayed based on biological process, molecular functions, and cellular components.

4. Discussion

Blue mold decay caused by *P. expansum* is a serious disease in fruit, in particular pear fruits [6]. Recently, some pathogenic strains developed resistance against synthetic fungicides; therefore, researchers concern about environmental and food safety. Antagonistic yeasts are gaining considerable attention due to their beneficial environmental and food safety characteristics and also controlling postharvest diseases in fruits. Integrating transcriptomic and proteomic data to achieve meaningful insights into *P. expansum* inhibited by *M. guilliermondii* has rarely been reported. Therefore, the present work highlights the protein expression profile and transcriptomic changes of *P. expansum* in order to explore the molecular inhibitory mechanism of *M. guilliermondii*. GO categorization was compared between differentially expressed proteins from 2-DE and differentially expressed genes from RNA-Seq analysis. A majority of differentially expressed proteins and genes were involved in secondary metabolite synthesis, ATP synthesis, cellular basal metabolism, environmental immune response, genetic information processing, and metabolism.

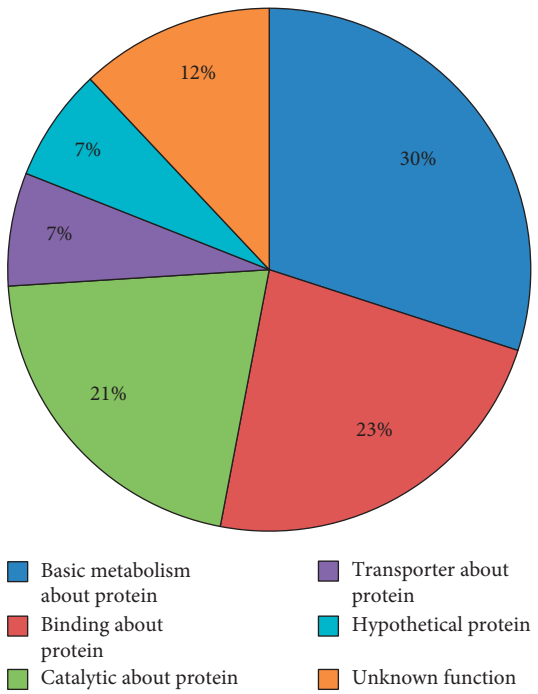

Figure 3: Functional classification of differentially expressed proteins in *P. expansum* after different treatments.

There are many enzymes responsible for synthesis of ATP; ATPase was considered as the most important enzyme in ATP synthesis. In the current investigation, protein ATPase, F1 complex (spots 17 and 38) corresponding with *ATPase, F0/V0 complex, subunit C* and *ATPase, F1/A1 complex, alpha subunit, N-terminal*, were all downregulated. Similar trend was observed in transcriptomic analysis; the gene corresponding to *ATPase*, *F0/V0 complex*, *subunit C* (TRINITY_DN3524_c0_g2, TRINITY_DN7527_c0_g1) was downregulated significantly. Yang et al. reported that the protein related to ABC transporters (PAAT), which is a novel ATPase and a *trans*-regulator of mitochondrial ABC transporters, is responsible for cell survival and maintenance of mitochondrial homeostasis [15]. Additionally, Yang et al. reported that the poplar buds active fraction (PBAF) depresses ATPase activity of *Penicillium italicum* and explained that the inhibitory effect of PBAF on fungal growth is due to the inhibition of ATPase by PBAF [16]. Castellote et al. investigated the molecular expression of *Geotrichum candidum* overripening of Reblochon-type cheese and observed that the genes responsible for F1F0 ATP synthase subunits (ATP1, ATP2, ATP5, and ATP7) were quiescent which leads to the reduced ATP production and consumption, normally less energy needed for quiescent cells [17]. From these reports, we anticipated that ATPase did not only provide energy for the basic metabolism of *P. expansum*, but also was the main carrier for the life activities of *P. expansum*. In the present investigation, the ATP activity of *P. expansum* treated with *M. guilliermondii* was reduced; thus, the energy supply was prevented and the growth was inhibited.

Heat shock protein 70 (HSP70) was a main part of the cell's machinery for protein folding and contributed to protection of cells against stress [18, 19]. In addition to the involvement of HSP70 in maintaining and improving the protein integrity, it directly inhibits apoptosis [20]. In our results, the heat shock 70 kDa protein (point 24) corresponded with the transcriptome genes *Heat, type 2* (TRINITY_DN5224_c0_g4), and both were downregulated. The intracellular heat shock protein and gene expression of *P. expansum* was downregulated because of the effect of *M. guilliermondii*. Zhang et al. studied the underlying molecular mechanisms of HSP70 in the environmental stress response of coral through transcriptome expression and reported that HSP70 (PdHSP70) was an essential stress regulatory protein in the stony coral *Pocillopora damicornis*; therefore, diverse environmental stress could induce HSP70 mRNA expression and its activity could remain stable under heat stress [21]. Kim et al. identified 10 Calmodulin (CaM)-binding protein in *Beauveria bassiana*; one of its targets was HSP70 and their results also suggested that ATP was involved in the inhibition of molecular interaction between CaM and HSP70 [22]. From the earlier studies, it was evidenced that downregulation of HSP70 weakens the adaptation ability and immune response of *P. expansum* to the external environment; the cells were apoptotic and the growth of *P. expansum* was inhibited by *M. guilliermondii*.

In biological systems, phosphoesterase plays a vital role in DNA fragmentation, RNA replication, human body medicine, metabolism, chemotherapy, and bioremediation [23, 24]. In the present results, the phosphoesterase protein (point 36) that corresponded with the transcriptome genes *phosphoesterase* (TRINITY_DN431_c0_g2) was downregulated. It could be speculated that the phosphate kinase activity of *P. expansum* was reduced and substance metabolism process was blocked due to the effect of *M. guilliermondii*, which resulted in shortage of phosphate needed for the survival of *P. expansum* and thus the normal life activity of *P. expansum* was inhibited by *M. guilliermondii*.

Polyketide synthases (PKSs) belong to a multidomain-enzyme or complex-enzyme family which are responsible for the production of polyketides, a major class of secondary metabolites, in microorganisms, plants, and some animal lineages [25, 26]. Polyketide synthases also play a definite role in the production of naturally occurring small molecules employed in chemotherapy [27]; many of the commonly used antibiotics and other industrially important polyketides were produced by polyketide synthases [28]. Our results demonstrated that the polyketide synthase, enoylreductase protein (point 20 and point 32), was downregulated. RNA-Seq analysis also showed that the gene responsible for polyketide synthases (TRINITY_DN10607_c0_g1) was downregulated significantly. Moreover, in *P. expansum*, the polyketide synthases are involved in the synthesis of patulin, a hazardous mycotoxin derived from polyketides [29]. Role of patulin in the pathogenicity and virulence of *P. expansum* was already proved in postharvest apples infected by *P. expansum* [30]. Patulin synthesis in *P. expansum* is carried out by a biosynthetic gene cluster consisted of 15 genes (*PatA-PatO*) in ten different enzymatic reactions [31]. Among them, the initial seven reactions in patulin synthesis are catalyzed by polyketide synthases [32]. Therefore, our

Table 2: Transcriptome sequencing data of *P. expansum* treated with *M. guilliermondii* (Y) and *P. expansum* (CK).

Samples	JSHY-ID	Read number	Base number	GC content (%)	%≥Q30
CK	T01	23336546	7000963800	54.33	89.75
Y	T02	20001194	6000358200	53.89	89.92

Table 3: The number of differentially expressed genes of *P. expansum* treated with *M. guilliermondii* (Y) and *P. expansum* (CK).

DEG set	DEG number	Upregulated	Downregulated
CK _vs._ Y	434	408	26

results may confirm that the polyketide synthase activity of *P. expansum* was reduced due to the inhibition of *M. guilliermondii*; thus the normal synthesis of patulin and other secondary metabolites of *P. expansum* was prevented. Likewise, *Ras-association* gene (TRINITY_DN6243_c0_g3) was also downregulated in *P. expansum* treated with *M. guilliermondii*. In most of the pathogenic fungi, Ras pathway signaling is determined as critical virulence factor [33]. The Ras pathway signaling is involved in pathogenesis, morphological transitions, nutrient sensing and acquisition, sexual reproduction, and stress responses of fungi [34]. The results also evidenced that the pathogenicity and virulence of *P. expansum* was weakened, and its growth was eventually inhibited by *M. guilliermondii*.

Phosphatidate cytidylyltransferase is an enzyme, involved in lipid transport and metabolism, that majorly takes part in the phospholipid metabolism in cell membranes. Lipids are essential components in membrane trafficking, cytoskeletal rearrangement, and secretion, which are reported to be the important mechanisms of stress tolerance [35]. In the present transcriptomic analysis, the gene responsible for *phosphatidate cytidylyltransferase* (TRINITY_DN3203_c0_g2) was upregulated in *P. expansum*. Bernardo et al. reported the upregulation and accumulation of phosphatidate cytidylyltransferase during drought stress in the roots of wheat [36]. Other than stress tolerance, phosphatidate cytidylyltransferase was also known for its role in antibiotic resistance mechanism against antimicrobial lipopeptides [37]. It was evidenced that in order to overcome the stress created by *M. guilliermondii* and to resist the activity of the yeast, the *phosphatidate cytidylyltransferase* gene was upregulated in *P. expansum*. In the same way, the upregulation of striatin, *N-terminal* (TRINITY_DN6439_c0_g1) was observed in the transcriptomic analysis. Striatin orthologs were directly related to the virulence of some filamentous fungi like *Fusarium verticillioides* and *F. graminearum* against plants [38]. Hence, we hypothesized that though some pathogenic related factors were inhibited by *M. guilliermondii*, some other genes related to stress tolerance and virulence were upregulated.

To sustain cellular redox homeostasis, the thioredoxin system assures a crucial function and the thioredoxin protein (point 2) was downregulated in the present study. Viefhues et al. indicated that thioredoxin had a strict influence on virulence of *Botrytis cinerea*, proving that redox processes were determinant for host-pathogen interactions in this pathogen; in addition, the balanced redox status established by the thioredoxin system was vital for growth and pathogenesis of *B. cinerea* [39]. Ianiri et al. investigated the modification of gene expression in *Sporobolomyces* sp. under patulin exposition; they noted the major increase in transcript levels of antioxidant molecules glutathione and thioredoxin in *Sporobolomyces* genes. The research team further subclassified the metabolic process of patulin exposed group and found that 6 DEGs were involved in glutathione biosynthesis, and 3 DEGs were involved in the thioredoxin system [40]. In contrast, by downregulation in the present study, we speculated that the thioredoxin system, which is involved in the defense response to ROS, was inhibited by *M. guilliermondii* and the growth of *P. expansum* was controlled.

The ribosomal S21 protein as part of eukaryotic ribosomes had proved to be strongly related to ribosome-associated protein in human and *Drosophila* cells [41–43]. In our results, the 40S ribosomal protein S21 (spot 4) was downregulated and was involved in nucleotide transport and metabolism. Sato et al. examined the functions of the ribosomal proteins S0 and S21 in *Schizosaccharomyces pombe* and found that the ribosomal protein played a major physiological role in 18s rRNA stability which was essential for the survival of *S. pombe* cells [44]. Therefore, we hypothesized that, in the presence of *M. guilliermondii*, 40S ribosomal protein S21 *P. expansum* was inhibited so that the growth and survival of the fungi was reduced dramatically.

Cytochromes P450 was distributed in almost all organisms and involved in diverse metabolic processes, which were highly diverse and contributed to the metabolism of xenobiotic compounds such as bioconversion of xenobiotics, biotransformation of drugs, and so on [45]. In the current investigation, the *Cytochrome P450* (TRINITY_DN5730_c0_g1) was upregulated. Trippe et al. studied the genetic underpinnings which permit *Graphium* sp. to catalyze the early step in the alkane and ether oxidation pathway; furthermore, they identified $CYP52L_1$ as an alkane-oxidizing Cytochrome P450, posttranscriptional ds-RNA-mediated gene silencing of $CYP52L_1$ and proved that the gene silencing disrupted the ability of *Graphium* sp. to grow on alkanes and ethers [46]. Similarly, our results revealed that the upregulation of *Cytochrome P450* (TRINITY_DN5730_c0_g1) could be activated by the basic metabolic process of *P. expansum* after it was cocultured with *M. guilliermondii*.

Glutathione S-transferases (GSTs) are well known for their capacity to reduce the oxidative potency of xenobiotic substrates for the purpose of detoxification [47, 48]. The main role of GSTs is to reduce the toxicity of xenobiotics compounds by catalyzing the GSH nucleophilic attack on electrophilic atoms of said nonpolar xenobiotic substrates, thereby avoiding their interaction with essential cellular proteins as well as nucleic acids [49]. In the present study, the *Glutathione S-transferase* (TRINITY_DN8139_c0_g1)

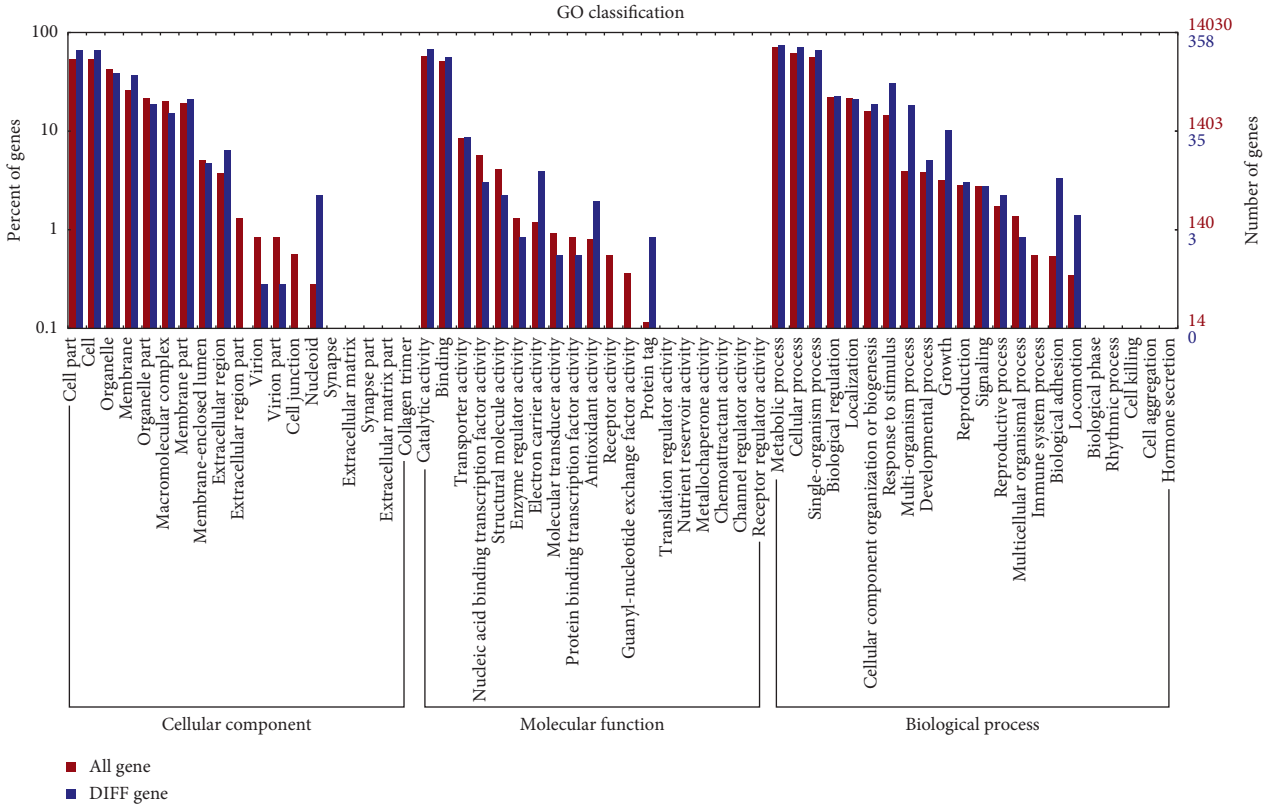

Figure 4: GO categorization of differentially expressed genes in *P. expansum*.

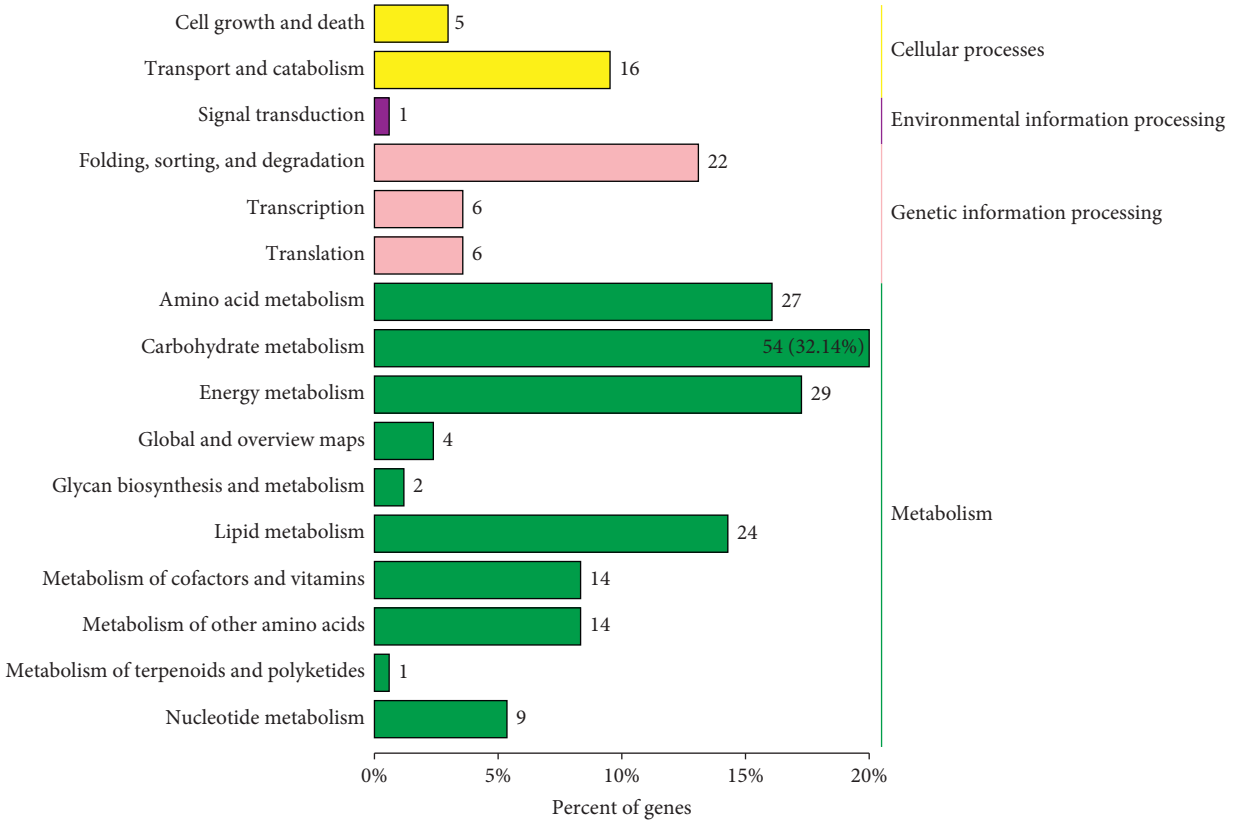

Figure 5: KEGG categorization of differentially expressed genes in *P. expansum*.

Table 4: Differentially expressed genes of *P. expansum* treated with *M. guilliermondii* (Y) and *P. expansum* (CK). FDR< 0.05 and $\log_2$ fold change ≥2 were considered statistically significant.

Functional classification	Gene ID	Gene annotation	Fold change	FDR
Secondary metabolites biosynthesis, transport, and catabolism	TRINITY_DN10607_c0_g1	*Polyketide synthase*	−9.638	0.0144304
ATP hydrolysis coupled proton transport	TRINITY_DN3524_c0_g2	*ATPase, F0/V0 complex, subunit C*	−9.654	0.0498454
ATP hydrolysis coupled proton transport	TRINITY_DN7527_c0_g1	*ATPase, F1/A1 complex, alpha subunit, N-terminal*	−9.882	0.0450140
Signal transduction mechanisms	TRINITY_DN6243_c0_g3	*Ras-association*	−10.069	0.0414577
Cell wall/membrane/envelope biogenesis	TRINITY_DN431_c0_g2	*Phosphoesterase*	−10.667	0.0296615
Cell organelle organization	TRINITY_DN5224_c0_g4	*HEAT, type 2*	−10.048	0.0415417
Ascospore formation	TRINITY_DN6439_c0_g1	*Striatin, N-terminal*	12.128	0.0144304
Lipid transport and metabolism	TRINITY_DN3203_c0_g2	*Phosphatidate cytidylyltransferase*	10.235	0.0381777
Secondary metabolites biosynthesis, transport, and catabolism	TRINITY_DN5730_c0_g1	*Cytochrome P450, E-class, group*	13.040	0.0138044
Posttranslational modification, protein turnover, chaperones	TRINITY_DN8139_c0_g1	*Glutathione S-transferase/chloride channel, C-terminal*	11.707	0.0138044

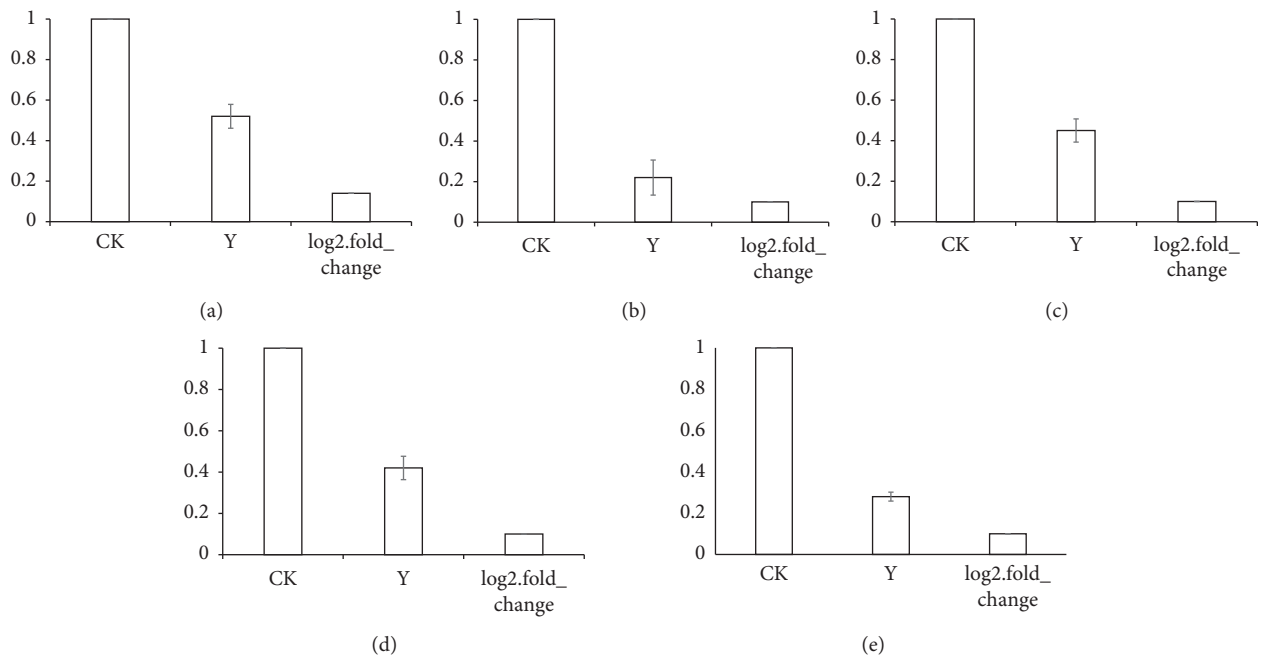

Figure 6: Verification of differentially expressed genes by RT-qPCR. Treatments: CK: *P. expansum* treated with sterile distilled water, Y: *P. expansum* treated with *M. guilliermondii*. Values are means of two independent experiments. Error bars represent the standard error of the mean. (a) Phosphoesterase. (b) Polyketide synthase, enoylreductase. (c) ATPase, F0/V0 complex, subunit C. (d) ATPase, F1/V1 complex, alpha subunit, N-terminal. (e) HEAT, type 2.

was upregulated. Ouyang et al. showed that citral possibly triggered a reduction in the mitochondrial membrane potential (MMP), intracellular ATP, and glutathione content, in contrast to an increase in the glutathione S-transferase activity and the accumulation of reactive oxygen species (ROS); these results indicated that the addition of citral probably leads to the oxidative damage of *Penicillium digitatum* and inhibited the growth of *P. digitatum* [50]. Hence, from the upregulation of *GST*, it could be postulated that the GST activity was activated in order to overcome the increased ROS in the presence of *M. guilliermondii*. The results also evidenced that *M. guilliermondii* created oxidative stress against *P. expansum*; hence, the level of GST (antioxidative enzyme) was increased. Overall, we have hypothesized that though some stress tolerance mechanism of *P. expansum* was activated, *M. guilliermondii* inhibit the growth and pathogenesis of *P. expansum* by downregulating certain pathways of virulence mechanism, cellular signal transduction, mycotoxin production, and cell organization.

On the other hand, the biocontrol efficacy of *M. guilliermondii* and pathogenic ability of *P. expansum* might be altered under availability of different nutritional sources

such as carbon (C), nitrogen (N), and environmental factors such as pH value, temperature, and water activity. Jianjie et al. studied the effect of nutrition and environmental factors on the biocontrol potential of *Esteya vermicola* and reported that both of them have great influence on the biocontrol potential [51]. Similarly, the growth and mycotoxin producing ability of *P. expansum* was altered by different glucose-containing sugars, complex N sources, and acidic conditions were favorable conditions for patulin production [52]. Since the stress tolerance of *M. guilliermondii* in extreme salinity stress was proved already [53], we speculated that the antagonistic potential of *M. guilliermondii* might be worthy in a diverse range of nutritional supplements and environmental factors. However, in the future prospect, the effect of various nutritional sources and environmental factors in the biocontrol mechanism of *M. guilliermondii* are needed to be studied.

In conclusion, several genes, such as *HEAT*, *Phosphoesterase*, *Polyketide synthase,* and *ATPase*, disclosed a corresponding relationship between the transcript and protein levels and revealed that those genes could play an important role in the growth regulation of *P. expansum*. Moreover, the data from transcriptomic and proteomic analysis discussed in the present study have not only underlined a set of genes and proteins that were essential in the growth of *P. expansum*, but also gave fundamental knowledge of molecular mechanism behind the inhibitory activity of *M. guilliermondii* against the growth of mold.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors have no conflicts of interest to declare.

Acknowledgments

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Supplementary Materials

Supplementary Table 1: primer design of basal metabolism and response regulation genes in P. expansum. (*Supplementary Materials*)

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