Article

The C2H2 Zinc Finger Protein MaNCP1 Contributes to Conidiation through Governing the Nitrate Assimilation Pathway in the Entomopathogenic Fungus Metarhizium acridum

Chaochuang Li 1,2,3, Yuxian Xia 1,2,3,* and Kai Jin 1,2,3,*

1 Genetic Engineering Research Center, School of Life Sciences, Chongqing University, Chongqing 401331, China
2 Chongqing Engineering Research Center for Fungal Insecticide, Chongqing 401331, China
3 Key Laboratory of Gene Function and Regulation Technologies Under Chongqing Municipal Education Commission, Chongqing 401331, China
* Correspondence: yuxianxia@cqu.edu.cn (Y.X.); jinkai@cqu.edu.cn (K.J.); Tel.: +86-23-6512-0990 (K.J.)

Abstract: Zinc finger proteins are an important class of multifunctional regulators. Here, the roles of a C2H2 zinc finger protein MaNCP1 (Metarhizium acridum nitrate-related conidiation pattern shift regulatory factor 1) in nitrogen utilization and conidiation were explored in the entomopathogenic fungus M. acridum. The results showed that MaNCP1-disruption mutant (∆MaNCP1) impaired the ability to utilize nitrate, ammonium and glutamine and reduced the expression of nitrate assimilation-related genes, suggesting that MaNCP1 was involved in governing nitrogen utilization. In addition, the conidial yield of the ∆MaNCP1 strain, cultured on the microcycle conidiation medium (SYA), was significantly decreased, which could be restored or even enhanced than that of the WT strain through increasing the nitrate content in SYA medium. Further study showed that MaAreA, a core regulator in the nitrogen catabolism repression (NCR) pathway, was a downstream target gene of MaNCP1. Screening the differential expression genes between WT and ∆MaNCP1 strains revealed that the conidial yield of M. acridum regulated by nitrate might be related to NCR pathway on SYA medium. It could be concluded that MaNCP1 contributes to the nitrate assimilation and conidiation, which will provide further insights into the relationship between the nitrogen utilization and conidiation in fungi.

Keywords: Metarhizium acridum; C2H2 zinc finger protein MaNCP1; nitrate assimilation; conidiation; MaAreA

1. Introduction

Crop pests seriously threaten agricultural production, and are important factors restricting global food production and quality, causing huge losses to agriculture every year [1,2]. Therefore, the effective control of crop pests is the important link in food security production [3,4]. Entomopathogenic fungi are the natural pathogenic microorganisms of insects and exhibit great potentials in agricultural pest control. For example, Beauveria bassiana and Metarhizium anisopliae have been developed into pesticides and applied in pest control [5]. For fungal pesticides, conidia are the main effective components to adhere to host cuticles, and germinate under certain conditions to form appressoria, then forming infection pegs to penetrate host cuticles and enter into hemolymph, and finally destroying immune systems and killing host pests [6]. Conidial production is one of the main restrictive factors for the large-scale application of fungal pesticides [7,8]. Thus, elucidating the regulatory mechanism of conidiation in entomopathogenic fungi will be helpful to fully tap their potentials in agricultural pest control.

Asexual sporulation is the main reproductive mode of filamentous fungi. At present, numbers of sporulation-related genes have been characterized, BrlA, AbaA, and WetA are the
central regulatory genes in the conidiation regulatory pathway in *Aspergillus nidulans* [9–11], mutations in any of these three regulatory factors will block the development of conidia [9]. *fluG*, *flbA*, *flbB*, *flbC*, *flbD* and *flbE* play positive roles in regulating the central regulatory pathway, which were known as the upstream developmental activators (UDAs), and are mainly to activate *brlA* and initiate conidial formation [12,13]. *fluG*, locates at the most upstream of UDAs, is a key activator for the initiation of conidia in *A. nidulans* [14]. In fact, asexual sporulation can be affected by many factors, among which nutritional condition, such as nitrogen source [15–17]. However, the relationship between the nitrogen utilization and conidiation in fungi is not fully understood in fungi.

For most filamentous fungi, they also have a further conidiation pattern, that is, microcycle conidiation [18]. Previous results have shown that the microcycle conidiation medium (SYA) can induce insect pathogenic fungus *M. acridum*, an acridid-specific pathogen, to perform microcycle conidiation [19], which would promote the fungal conidiation and increase the conidial yield, and these conidia exhibited higher thermostolerance and similar UV-B tolerance and virulence [20]. Meanwhile, increasing the nitrate in SYA medium could shift the conidiation pattern in *M. acridum* [21], suggesting that nitrate metabolism may play important roles during this process.

In fungi, nitrate assimilation plays important roles in nitrate utilization and is regulated by the NCR path, which is mediated by GATA transcription factors [22]. *AreA* and *AreB*, the only two GATA factors in NCR path are characterized in filamentous *Ascomycetes*, and the core transcriptional activator *AreA* could work synergistically with *NirA* to promote nitrate assimilation in *A. nidulans* [23]. Under nitrogen starvation conditions, higher areA transcription levels [24], more stable mRNA levels [25,26] and preferential nuclear localization of protein [27] would increase the abundance and activity of *AreA*. Furthermore, *AreA* could interact with the negative regulatory factor *NmrA* to decrease the abundance and activity of *AreA* when the nitrogen is sufficient [28,29].

Recently, *MaNCP1* contributes to the conidiation pattern via nitrate assimilation was identified [30] and its N-terminal zinc fingers make contributions to the fungal growth and virulence in *M. acridum* [31]. However, the roles of *MaNCP1* in conidiation need to be further explored. Here, we focused on the relationship between nitrate assimilation and conidiation. These data revealed that *MaNCP1* contributes to the conidiation through governing the nitrate assimilation pathway, which will broaden the research field of nutritional regulation conidiation in filamentous fungi.

2. Materials and Methods

2.1. Strains and Culture Conditions

All *MaNCP1*-related mutants have been generated in our previous study [30], and cultured on 1/4SDAY (2.5‰ peptone, 5‰ yeast extract, 10‰ glucose and 18‰ agar, w/v), SYA (30‰ sucrose, 5‰ yeast extract, 0.5‰ MgSO$_4$, 1‰ KH$_2$PO$_4$, 0.5‰ KCl, 0.01‰ MnSO$_4$, 0.01‰ FeSO$_4$, 3‰ NaNO$_3$ and 18‰ agar, w/v), SYA+N (SYA supplemented with 40.5‰ NaNO$_3$, w/v), Czapek-dox (CZA) (0.01‰ FeSO$_4$, 0.5‰ KCl, 0.5‰ MgSO$_4$, 1‰ K$_2$HPO$_4$, 2‰ NaNO$_3$, 30‰ sucrose and 18‰ agar, w/v) and/or modified CZA medium at 28 °C for days. The *M. acridum* strain CQMa102 (wild-type, WT) was deposited in China General Microbiological Culture Collection Center (CGMCC, Beijing, China; No. 0877). *Escherichia coli* DE3 (Solarbio, Beijing, China) was used for the prokaryotic expression of the target proteins. Y187 (Clontech, Palo Alto, CA, USA) yeast strain was used for yeast one-hybrid assay.

2.2. Conidial Yield and Fungal Growth Assays

Conidial suspensions with a concentration of 10$^6$ conidia/mL were prepared after the fungal strains grown on 1/4SDAY for 15 d, pipetting 2 μL and, respectively, inoculated into 24-cellplates contain SYA, SYA + N, CZA or modified CZA media and followed by growing at 28 °C to determinate the conidial yield [32]. Two microliters conidial suspensions were,
respectively, inoculated into CZA or modified CZA with different nutrients and cultured at 28 °C for 7 d to observe the growth of each fungal strain.

2.3. Bioinformatics Analysis and Yeast One-Hybrid Assay

The putative binding cis-element of MaNCP1 was analyzed with JASPAR 2020 [33]. The cDNA of MaNCP1 and the promoter sequence of MaAreA-1 (−2019~−996) and MaAreA-2 (−1013~+51) were ligated into pGADT7 or pHIS2 vector to generate pGADT7-MaNCP1, pHIS2-MaAreA-1 and pHIS2-MaAreA-2, respectively. 3-amino-1,2,4-triazole (3-AT), a competitive inhibitor, was added into selected medium SD/-Trp/-His (DDO) to inhibit the leakage expression of HIS3. Co-transforming pGADT7-MaNCP1 with pHIS2-MaAreA-1/2 into Y187 to obtain Y187 (pGADT7-MaNCP1 × pHIS2-MaAreA-1/2), then spread on SD/-Leu/-Trp/-His (TDO) and TDO contained 3-AT with the control group setting: positive controls (pGADT7-53 and pHIS2-53) and negative control (pGADT7-MaNCP1 and pHIS2).

2.4. Electrophoretic Mobility Shift Assay (EMSA)

The expression and purification of the N-terminal of MaNCP1 protein (228 aa) were performed according to our previously study [30]. The DNA probe was cloned with primer pairs AreA-P-F/AreA-P-R (Supplementary Table S1). EMSA Probe Biotin Labeling Kit (Beyotime, Shanghai, China) and Chemiluminescent EMSA kit (Beyotime, Shanghai, China) were used for labeling the probe with biotin and EMSA, respectively [30].

2.5. Quantitative Reverse Transcription PCR (qRT-PCR) Analyses

Fungal strains, cultured on SYA or SYA+N plates at 28 °C for 12, 18 or 24 h, were harvested and used for extracting RNA with Fungal RNA Kit (OMEGA, USA), followed by synthesizing cDNA via PrimeScript™ RT reagent Kit with gDNA Eraser (TaKaRa, Dalian, China), and qRT-PCR were performed with SYBR® Premix Ex Taq™ kit (TaKaRa, Dalian, China). Data were analyzed with the 2−ΔΔCt method [34] with the internal control gene gpdh (MAC_09584) and the glyceraldehyde 3-phosphate dehydrogenase gene. Primers were listed in Supplementary Table S1.

2.6. RNA-Seq Analysis

RNA-seq was performed to uncover the regulatory roles MaNCP1 involved in nitrate metabolism. Fungal RNAs were extracted after the strains growing on SYA+N media at 28 °C for 18 h, and then submitted to BGISEQ-500 sequencing platform (BGI, Shenzhen, China) for RNA-Seq with three biological replicates. Genes with \( \log_2(\Delta \text{MaNCP1}_\text{N}/\text{WT}_\text{N}) \geq 1 \) and FDR (false-discover rate) \( \leq 0.001 \) were defined as the DEGs.

2.7. Statistical Analysis

The data (mean ± SE) were analyzed using the SPSS 24.0 via ANOVA (one-way analysis of variance) or T-text method with three biological replicates.

3. Results

3.1. MaNCP1 Regulates the Conidiation

Analysis of the conidial yield of the fungal strains under SYA or SYA+N conditions found that it was significantly decreased in the absence of MaNCP1 under SYA condition, which could be restored by increasing exogenous nitrate into the SYA medium (i.e., the SYA + N medium) and finally higher than that of the WT (Figure 1), suggesting that the conidiation was mediated by nitrate.

3.2. MaNCP1 Regulates the Nitrogen Utilization

The colony of ΔMaNCP1 that grown on the nitrate or Glu was slightly smaller than that of the WT or CP (complementary) strains, which was significantly larger when Gln as the sole nitrogen source, but with no difference when grown on the \((\text{NH}_4)_2\text{SO}_4\) medium.
The conidial yield of the ∆MaNCP1 strain cultured on nitrate, (NH₄)₂SO₄, Gln or Glu media were all significantly reduced, whether they were cultured for 7 d or 15 d (Figure 2C). Interestingly, the color of the media around the colony of ∆MaNCP1 strain changed to yellow at the 7 day when Gln was used as the nitrogen source, whether culturing on the plates (Figure 2A) or inoculating into the 24-cell plates, in which the growth of the colony was severely inhibited (Figure 2D). Furthermore, the color of the fermentation broth that inoculated with ∆MaNCP1 strain cultured for 48 h were also changed to yellow (Figure 2D), and the biomass was significantly reduced (Figure 2E). These results indicated that MaNCP1 was involved in the regulation of nitrogen utilization.

**Figure 1.** Conidial yields of the WT and ∆MaNCP1 strains under SYA or SYA+N conditions. SYA+N, SYA supplemented with sodium nitrate.

**Figure 2.** MaNCP1 affects the nitrate assimilation. Colony morphology (A) and colony areas (B) of the fungal strains grown on the modified CZA medium at 28 °C for 7 days. The concentration of all nitrogen sources was 10 mM. (C) Conidial yield of each strain grown on the modified CZA medium and cultured for 7 or 15 d, respectively. (D) Colony morphology of each strain cultured in the 24-well plates for 7 d (Top), and fermentation liquor of the fungal strains inoculated in CZB (CZA without agar) with 220 rpm for 48 h (Bottom). Gln (10 mM) was acted as the sole nitrogen source in CZB liquid medium. (E) Biomass of the liquid fermentation for 48 h. Gln, Glutamine. Glu, Glutamate. Lowercase letters indicate significant difference at p < 0.05 (Tukey’s HSD at the same treatment and the letters indicate a comparison between strains cultivated on the same modified CZA.
3.3. MaNCP1 Regulates the Expression of Nitrate Metabolism Genes

To further explore the function of MaNCP1 in nitrate assimilation, nine genes, including nitrate transporter gene MaNrtB (MAC_03189), nitrate reductase gene MaNR (MAC_08624), nitrite reductase gene MaNiR (MAC_03493), glutamine synthetase 1 gene MaGS1 (MAC_01108), glutamine synthetase 2 gene MaGS2 (MAC_06858) and glutamine synthetase 3 gene MaGS3 (MAC_04461), glutamate synthase gene MaGOGAT (MAC_00032), glutamate dehydrogenase 1 gene MaGDH1 (MAC_08384) and glutamate dehydrogenase 2 gene MaGDH2 (MAC_01648), in the nitrate metabolism pathway were searched out from M. acridum genome. Under SYA culture conditions (Figure 3A), the expression of MaNrtB in ∆MaNCP1 background was significantly down-regulated, indicating that the transmembrane transport of nitrate from extracellular to intracellular was impaired with disruption of MaNCP1. The transcription of MaNR, MaNiR, MaGS1 and MaGS2 were all down-regulated, MaGDH1, MaGDH2 and MaGOGAT were all up-regulated at 12 h or 18 h, MaGS3 was down-regulated at 12 h or 18 h. Under SYA+N culture conditions (Figure 3B), genes, except for MaNiR and MaGS1, were all up-regulated to varying degrees at different time points in ∆MaNCP1 background, indicating that MaNiR and MaGS1 were MaNCP1-dependent expression under SYA + N condition. It confirmed that MaNCP1 was involved in regulating nitrate assimilation pathway.

Figure 3. MaNCP1 regulated the expression of nitrate and ammonium assimilation genes. Transcription level analysis of genes in nitrate and ammonium assimilation pathway under SYA (A) and SYA + N (B) conditions. Fungal strains were cultured on SYA and/or SYA+N media for 12, 18 or 24 h. Lowercase letters indicate significant difference at $p < 0.05$ (T-text).
3.4. MaAreA Is a Target Gene of MaNCP1

AreA is known to play important roles in nitrate metabolism. Bioinformatics analysis found the MaAreA promoter contained the recognition site of MaNCP1 (Figure 4A). In addition, the transcription level of MaAreA (MAC_00939) in ΔMaNCP1 background was significantly down-regulated under both SYA and SYA+N conditions (Figure 4B), indicating that MaAreA was a MaNCP1-dependent expression gene. Furthermore, the Y187 (pHIS2-MaAreA-1) and Y187 (pHIS2-MaAreA-2) yeast strains did not grow on the DDO plate with 8 mM 3-AT (Supplementary Figure S2), and only the co-transformed strain Y187 (pGADT7-MaNCP1 × pHIS2-MaAreA-1) could grow normally on the TDO + 3-AT (8 mM) plate (Figure 4C). Moreover, EMSA confirmed that the N-terminal of MaNCP1 protein could bind to MaNmrA (Figure 4D). These results showed that MaAreA was a downstream target gene of MaNCP1.

![Figure 4](image-url)

**Figure 4.** MaAreA is a target gene of MaNCP1. (A) Putative binding site prediction of MaNCP1. The potential cis-element was identified via JASPAR 2020 database [33]. (B) Transcription level analysis of MaAreA. Fungal strains were spread on SYA or SYA+N media and cultured at 28 °C for 18 h. Lowercase letters indicate significant difference at \( p < 0.05 \) (T-text). (C) Yeast one-hybrid assay. TDO, SD/-Leu/-Trp/-His. The concentration of 3-AT was 8 mM. (D) The EMSA analysis. MBP, MBP-tag protein. MaNCP1-N, the N-terminal of MaNCP1 protein. Competitor, the unlabeled probe, which was added in a 100-fold excess. +, probe or protein added, −, probe or protein not added.

3.5. Transcriptomic Insights into Pleiotropic Effects of MaNCP1

To identify the genes and pathways that regulated by MaNCP1, RNA-seq was performed and a total of 9,699 genes were mapped to the genome, and 72 DEGs (up/down ratio, 43:29) were identified (Figure 5A; Supplementary Table S2). To verify the dependability of the RNA-seq data, 22 DEGs were randomly selected to detect their expression by qRT-PCR. As a result, the expression patterns of all these DEGs were similar to those from the RNA-seq data (Supplementary Figure S1). The Gene Ontology (GO) function an-
notation showed that these DEGs were mostly enriched to biological process and involved mainly in metabolic process, cellular process, localization, response to stimulus, biological regulation, regulation of biological process and signaling; enriched to cellular component and involved mainly in membrane, membrane part, cell and cell part; enriched to molecular function and involved mainly in catalytic activity, binding and transporter activity (Figure 5B). Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis showed that these DEGs enriched to 13 pathways, such as cellular community-prokaryotes, transcription, translation, amino acid metabolism, carbohydrate metabolism, energy metabolism, global and overview maps, glycan biosynthesis and metabolism, lipid metabolism, metabolism of cofactors and vitamins, metabolism of other amino acids, nucleotide metabolism and xenobiotics biodegradation and metabolism (Figure 5C), suggesting that \textit{MaNCP1} was involved in multiple biological and metabolic regulatory processes.

![Figure 5. RNA-seq analysis of Δ\textit{MaNCP1} \textit{N-vs-WT} \textit{N}. (A) Distributions of DEGs. (B) Analysis of the GO function classes. (C) KEGG pathway classification of DEGs. (D) Clustering analysis of the known DEGs.](image)
In addition, among these DEGs, 19 genes (up/down ratio, 11:8) encode hypothetical proteins, the other 53 genes were annotated, in which 4 genes were directly involved in nitrogen metabolism (MAC_05898, MAC_04042, MAC_09768 and MAC_07959), 6 genes were involved in sugar metabolism (MAC_07957, MAC_09405, MAC_02819, MAC_00175, MAC_01184 and MAC_02571), 9 genes were involved in pathogenicity (MAC_07957, MAC_05899, MAC_06622, MAC_06606, MAC_06944, MAC_08413, MAC_05384, MAC_08685 and MAC_05385), 6 genes were involved in growth and development (MAC_07957, MAC_06622, MAC_02834, MAC_06944, MAC_09145 and MAC_08685) and 3 genes were involved in stress tolerance (MAC_07958, MAC_04217 and MAC_05385) (Supplementary Table S3). Further analysis revealed that MAC_09768 (Figure 5D), a DEG involved in the RNA-seq data, encoding an amino acid permease, which was homologous to Saccharomyces cerevisiae amino acid permease GAP1 (NP_012965.3). GAP1 is an important marker protein to respond to NCR and a main regulator of yeast plasma membrane in nitrogen starvation condition [35,36], and could interact with AreA in A. nidulans [37]. Therefore, we reasoned that the involvement of MaNCP1 in regulating conidiation may be related to the NCR pathway.

4. Discussion

Zinc finger proteins are an important class of multifunctional regulators, in which the C2H2 zinc finger proteins have been reported in many fungi and play crucial roles in the regulation of conidiation, such as amdX [38] and nsdC [39] in A. nidulans, StuA in Acremonium chrysogenum [40], PacC [41] and Msn2 [42] in M. acridum. Previous studies have shown that MaNCP1 regulates the conidiation pattern [30] and virulence in M. acridum [31]. MaNCP1 homologous gene MGG_07339 regulates the conidiation and virulence in Magnaporthe oryzae [43]. In this study, we revealed to the role of MaNCP1 in the regulation of conidiation by affecting nitrate assimilation in M. acridum.

Previous study showed that MaNCP1 regulates the nitrogen metabolism, and the DEGs of WT vs. ΔMaNCP1 (grown on SYA media) showed that 10 genes (MAC_00595, MAC_02196, MAC_02717, MAC_04470, MAC_05894, MAC_06018, MAC_07958, MAC_07959, MAC_09703 and MAC_00141) among the 45 annotated DEGs are involved in amino acid metabolism, which encoding cytochrome P450, NmrA family transcriptional regulator, catalase, oxidoreductase, major facilitator superfamiliy protein, tyrosinase, hydan-toinase/oxoprolinase, FAD binding domain-containing protein and hypothetical protein, respectively [30]. Here, we had shown that the change in conidial yield caused by MaNCP1 was reversible by increasing the nitrate content in SYA medium, suggesting that the conidiation mediated by MaNCP1 was related to nitrate metabolism. Moreover, the transcription levels of genes related to the nitrate assimilation pathway were all up- or down-regulated in ΔMaNCP1 background. Furthermore, our results also showed that MaNCP1 affected the utilization of nitrate, ammonium, Gln and Glu, and the utilization of Gln may cause the change in secondary metabolites, resulting in the color change in the solid medium or liquid fermentation broth that inoculated with ΔMaNCP1 strain. RNA-seq analysis under SYA+N condition showed that MaNCP1 involved in multiple metabolic pathways, the amino acid permease MAC_09768, a DEG, is homologous to the amino acid permease GAP1 in S. cerevisiae, which is regulated by the Gln3 and Gat1 in S. cerevisiae [44]. It has been reported that the GATA transcription factor AreA, which function is similar to Gln3/Gat1, interacts with Gap1 in A. nidulans [37]. In this study, we confirmed that MaAreA was a direct target gene of MaNCP1. Many studies have confirmed that AreA is a core regulator in nitrogen metabolism [45–49].

Furthermore, it is confirmed that MaNmrA is a further downstream target gene of MaNCP1 and its expression level is significantly down-regulated in ΔMaNCP1 background [30], which is similar to MaAreA. In A. nidulans, NmrA can interact with AreA to form NmrA-AreA heterodimer and inhibit the utilization of alternative nitrogen sources (i.e., nitrate and nitrite) under sufficiently nitrogen source conditions by modulating the activity of nitrate and/or nitrite reductase. If not, it will not form NmrA-AreA complex and promoting the utilization of alternative nitrogen sources [28,50]. Although study has
shown that SYA medium does not provide a classical NCR condition [30]. In general, the extremely significant down-regulation of MaNmrA will alleviates the inhibition on MaAreA in ΔMaNCP1 background. In addition, the additional nitrate did not induce the expression of MaAreA, and the expression of the core genes (MaNR and MaNiR) in nitrate assimilation were significantly reduced in the absence of MaNCP1 whether under SYA or SYA+N conditions. Under SYA condition, deletion of MaNCP1 significantly reduced the conidial yield, however, which could be restored when exogenous nitrate was added into SYA medium. Studies have shown that nitric oxide (NO), a by-product in nitrate metabolism, is involved in regulating conidiation in Neurospora crassa [51] and Coniothyrium minitans [52]. However, excessive NO accumulation can cause the cellular nitrooxidative stress, and our previous study shows that deletion of MaNCP1 can reduce the intracellular NO content and enhance the expression of the flavohemoglobin genes, MaFhb1 and MaFhb2 [30], which play indispensable roles in catalyzing the redox process of NO [53,54]. Taken together, our results showed that MaNCP1 may be involved in the NCR pathway by acting on MaAreA and MaNmrA to regulate nitrate metabolism and conidiation, suggesting that MaNCP1 play important homeostatic roles in fungal nitrate metabolism and nitrogen utilization.

5. Conclusions

In summary, the results of MaNCP1 regulating the conidiation in M. acridum will provide candidate genes and theoretical guidance for improving the conidial quality and yield to reduce the production cost and maintain the stable efficiency of mycoinsecticides.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/jof8090942/s1, Figure S1: The verification of DEGs by qRT-PCR. Figure S2: Screening of 3-AT background concentration of MaAreA promoter sequences. Y187(pHIS2-MaAreA-1) and Y187(pHIS2-MaAreA-2) were evenly spread on SD/-Trp/-His plate and SD/-Trp/-His plate with 8mM 3-AT to observe the growth of yeast. pGADT7-S3 and pHIS2-S3 as positive controls (PC), pGADT7-MaNCP1 and pHIS2 as negative control (NC). Table S1: Primers used in this study. Table S2: Differentially expressed genes (DEGs) in RNA-seq. Table S3: Differentially expressed genes involved in nutrition utilization, growth and development, stress tolerance and pathogenicity [55–79].

Author Contributions: Conceptualization, Y.X. and K.J.; Data curation, C.L.; Funding acquisition, K.J.; Investigation, C.L.; Methodology, Y.X. and K.J.; Project administration, K.J.; Software, C.L.; Writing—original draft, C.L.; Writing—review and editing, K.J. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the National Natural Science Foundation of China (32172479), the Venture & Innovation Support Program for Chongqing Overseas Returnees (cx2019035), Chongqing Talent Program (cstc2021ycjh-bgzxmx0313).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: RNA-seq data had been are deposited in the NCBI BioProject database (accession No. PRJNA799900).

Conflicts of Interest: The authors declare no conflict of interest.

References
1. Savary, S.; Bregaglio, S.; Willocquet, L.; Gustafson, D.; D’Croz, D.M.; Sparks, A.; Castilla, N.; Djurle, A.; Allinne, C.; Sharma, M.; et al. Crop health and its global impacts on the components of food security. Food Secur. 2017, 9, 311–327. [CrossRef]
2. Savary, S.; Willocquet, L.; Pethybridge, S.J.; Esker, P.; McRoberts, N.; Nelson, A. The global burden of pathogens and pests on major food crops. Nat. Ecol. Evol. 2019, 3, 430–439. [CrossRef] [PubMed]
3. Savary, S.; Teng, P.S.; Willocquet, L.; Nutter, F.W. Quantification and modeling of crop losses: A review of purposes. Annu. Rev. Phytopathol. 2006, 44, 89–112. [CrossRef] [PubMed]
4. Esker, P.; Savary, S.; McRoberts, N. Crop loss analysis and global food supply: Focusing now on required harvests. CAB Rev. 2012, 7, 1–14. [CrossRef]
5. Wang, C.S.; Feng, M.G. Advances in fundamental and applied studies in China of fungal biocontrol agents for use against arthropod pests. *Biol. Control* **2014**, *68*, 129–135. [CrossRef]
6. Wang, C.S.; Wang, S.B. Insect pathogenic fungi: Genomics, molecular interactions, and genetic improvements. *Annu. Rev. Entomol.* **2017**, *62*, 73–90. [CrossRef]
7. Jackson, M.A.; Cliquet, S.; Ilen, L.B. Media and fermentation processes for the rapid production of high concentrations of stable blastospores of the biocontrol fungus *Paecilomyces fumosoroseus*. *Biocontrol Sci. Techn.*** 2003, *13*, 23–33. [CrossRef]
8. Li, C.C.; Xia, Y.X.; Jin, K. N-terminal zinc fingers of MaNCP1 contribute to growth, stress tolerance, and virulence in entomopathogenic fungus *Metarhizium acridum*. *Curr. Genet.* **2012**, *58*, 877–888. [CrossRef] [PubMed]
9. Li, C.C.; Zhang, Q.P.; Xia, Y.X.; Jin, K. MaNmrA, a negative transcription regulator in nitrogen catabolite repression pathway, contributes to nutrient utilization, stress resistance and virulence in entomopathogenic fungus *Metarhizium acridum*. *Microbiol. Mol. Biol. Rev.* **2012**, *76*, 599–619. [CrossRef] [PubMed]
33. Fornes, O.; Castro-Mondragon, J.A.; Khan, A.; van der Lee, R.; Zhang, X.; Richmond, P.A.; Modi, B.P.; Corread, S.; Gheorghe, M.; Baranasic, D.; et al. JASPAR 2020: Update of the open-access database of transcription factor binding profiles. *Nucleic Acids Res.* 2020, 48, D87–D92. [CrossRef] [PubMed]

34. Livak, K.J.; Schmittgen, T.D. Analysis of relative gene expression data using real-time quantitative PCR and the 2^−ΔΔCT method. *Methods* 2001, 25, 402–408. [CrossRef] [PubMed]

35. Chen, E.J.; Kaiser, C.A. Amino acids regulate the intracellular trafficking of the general amino acid permease of *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA* 2002, 99, 14837–14842. [CrossRef] [PubMed]

36. Scherens, B.; Feller, A.; Vierendeels, F.; Messenguy, F.; Dubois, E. Identification of direct and indirect targets of the Gln3 and GAT1 activators by transcriptional profiling in response to nitrogen availability in the short and long term. *FEMS Yeast Res.* 2006, 6, 777–791. [CrossRef]

37. Cruz-Leite, V.R.M.; Salem-Izacc, S.M.; Novaes, E.; Neves, B.; Brito, W.D.; Silva, L.O.S.; Paccez, J.D.; Parente-Rocha, J.A.; Pereira, M.; Soares, C.M.D.; et al. Nitrogen Catabolite Repression in members of *Paracoccidioides* complex. *Microb. Pathog.* 2020, 149, 104281. [CrossRef] [PubMed]

38. Murphy, R.L.; Andrianopoulos, A.; Davis, M.A.; Hynes, M.J. Identification of amdx, a new Cys2His2 (C2H2) zinc-finger gene involved in the regulation of the amds gene of *Aspergillus nidulans*. *Microb. Mol. Biol. Rev.* 1997, 61, 591–602. [PubMed]

39. Kim, H.R.; Basheer, A.; Bock, S.; Reyes-Dominguez, Y.; Dalik, T.; Altmann, F.; Strauss, J. Dissecting individual steps of nitrogen catabolite repression in *Aspergillus nidulans* Geneticus 2009, 182, 771–783. [CrossRef] [PubMed]

40. Hu, P.; Wang, Y.; Zhou, J.; Pan, Y.; Liu, G. Acstua, which encodes an APSES transcription regulator, is involved in conidiation, cephalosporin biosynthesis and cell wall integrity of *Acremonium chrysogenum*. *Fungal Genet. Biol.* 2015, 83, 26–40. [CrossRef]

41. Zhang, M.; Wei, Q.; Xia, Y.; Jin, K. MaPacC, a pH-responsive transcription factor, negatively regulates thermotolerance and contributes to conidiation and virulence in *Metarhizium anisopliae*. *Curr. Genet.* 2020, 66, 397–408. [CrossRef]

42. Song, D.X.; Cao, Y.Q.; Xia, Y.X. Transcription factor MaMsn2 regulates conidiation pattern shift under the control of MaH1 through homeobox domain in *Metarhizium anisopliae*. *J. Fungi* 2021, 7, 840. [CrossRef]

43. Cao, H.; Huang, P.; Zhang, L.; Shi, Y.; Sun, D.; Yan, Y.; Liu, X.; Dong, B.; Chen, G.; Snyder, J.H.; et al. Characterization of 47 Cys2-His2 zinc finger proteins required for the development and pathogenicity of the rice blast fungus *Magnaporthe oryzae*. *New Phytol.* 2016, 211, 1035–1051. [CrossRef]

44. Stanbrough, M.; Magasanik, B. Two transcription factors, Gln3p and Nil1p, use the same GATAAG sites to activate the expression of GAP1 of *Saccharomyces cerevisiae*. *J. Bacteriol.* 1996, 178, 2465–2468. [CrossRef] [PubMed]

45. Narendja, F.; Goller, S.P.; Wolschuk, M.; Strauss, J. Nitrate and the GATA factor AreA are necessary for in vivo binding of NiaR, the pathway-specific transcriptional activator of *Aspergillus nidulans*. *Mol. Microbiol.* 2002, 44, 573–583. [CrossRef] [PubMed]

46. Muro-Pastor, M.I.; Strauss, J.; Ramon, A.; Scaccozio, C. A paradoxical mutant GATA factor. *Eukaryot Cell* 2004, 3, 393–405. [CrossRef]

47. Berger, H.; Basheer, A.; Bock, S.; Reyes-Dominguez, Y.; Dalik, T.; Altmann, F.; Strauss, J. Dissecting individual steps of nitrogen transcription factor cooperation in the *Aspergillus nidulans* nitrate cluster. *Microb. Mol. Biol. Rev.* 2008, 69, 1385–1398. [CrossRef] [PubMed]

48. Wong, K.H.; Hynes, M.J.; Todd, R.B.; Davis, M.A. Deletion and overexpression of the AreC gene encoding a putative C2H2-type transcription factor is a key activator of *Aspergillus nidulans* nitrate cluster. *Microb. Mol. Biol. Rev.* 2009, 73, 155, 3868–3880. [CrossRef] [PubMed]

49. Wiemann, P.; Sieber, C.M.K.; vonBargen, K.W.; Studt, L.; Niehaus, E.M.; Espino, J.J.; Huß, K.; Michielse, C.B.; Albermann, S.; Wagner, D.; et al. Deciphering the cryptic genome: Genome-wide analyses of the rice pathogen *Fusarium fujikuroi* reveals unexpected pleiotropy. *Eukaryot Cell* 2009, 8, D87–D92. [CrossRef] [PubMed]

50. Wilson, R.A.; Arst, H.N. Mutational analysis of AREA, a transcriptional activator mediating nitrogen metabolite repression in development and pathogenicity by the rice blast fungus *Magnaporthe oryzae*. *PLoS Pathog.* 2013, 9, e1003475. [CrossRef] [PubMed]

51. Ninnemann, H.; Maier, J. Indications for the occurrence of nitric oxide synthases in fungi and plants and the involvement in photocodination of *Neurospora crassa*. *Photoch. Photobiol.* 1996, 64, 393–398. [CrossRef]

52. Gong, X.; Fu, Y.; Jiang, D.; Li, G.; Yi, X.; Peng, Y. L-Arginine is essential for conidiation in the filamentous fungus *Conidiobolus minitans*. *Fungal Genet. Biol.* 2007, 44, 1386–1379. [CrossRef]

53. Zweier, J.L.; Li, H.; Samouilov, A.; Liu, X. Mechanisms of nitrite reduction to nitric oxide in the heart and vessel wall. *Nitric Oxide* 2010, 22, 83–90. [CrossRef] [PubMed]

54. Zhao, Y.; Lim, J.; Xu, J.; Yu, J.H.; Zheng, W. Nitric oxide as a developmental and metabolic signal in filamentous fungi. *Mol. Microbiol.* 2020, 113, 872–882. [CrossRef] [PubMed]

55. Badaruddin, M.; Holcombe, L.J.; Wilson, R.A.; Wang, Z.Y.; Kershaw, M.J.; Talbot, N.J. Glycogen metabolic genes are involved in trehalose-6-phosphate synthase-mediated regulation of pathogenicity by the rice blast fungus. *Magnaporthe oryzae*. *PLoS Pathog.* 2013, 9, e1003604. [CrossRef] [PubMed]

56. Zhou, T.; Qin, L.; Zhu, X.; Shen, W.; Zou, J.; Wang, Z.; Wei, Y. The D-lactate dehydrogenase MoDLD1 is essential for growth and infection-related development in *Magnaporthe oryzae*. *Environ. Microbiol.* 2017, 19, 3968–3978. [CrossRef]

57. Chen, W.; Wei, L.; Zhang, Y.; Shi, D.; Ren, W.; Zhang, Z.; Wang, J.; Shao, W.; Liu, X.; Chen, C.; et al. Involvement of the two L-lactate dehydrogenase in development and pathogenicity in *Fusarium graminearum*. *Curr. Genet.* 2019, 65, 591–605. [CrossRef]

58. Storts, D.R.; Bhattacharjee, J.K. Properties of revertants of lys2 and lys5 mutants as well as alpha-aminoadipate-semialdehyde dehydrogenase from *Saccharomyces cerevisiae*. *Biochem. Biophys. Res. Co.* 1989, 161, 182–186. [CrossRef]
59. Vander Beken, S.; de Vries, J.C.; Meier-Schiesser, B.; Meyer, P.; Jiang, D.; Sindrilaru, A.; Ferreira, F.F.; Hainzl, A.; Schatz, S.; Muschhammer, J.; et al. Newly defined ATP-binding cassette subfamily b member 5 positive dermal mesenchymal stem cells promote healing of chronic iron-overload wounds via secretion of interleukin-1 receptor antagonist. *Stem Cells* 2019, 37, 1057–1074. [CrossRef]

60. Koivistoinen, O.M.; Penttilä, M.; Ruohonen, L.; Mojzita, D. Sorbitol dehydrogenase of *Aspergillus niger*. SdhA, is part of the oxido-reductive D-galactose pathway and essential for D-sorbitol catabolism. *FEBS Lett.* 2012, 586, 378–383. [CrossRef]

61. Chang, T.S. An updated review of tyrosinase inhibitors. *Int. J. Mol. Sci.* 2009, 10, 2440–2475. [CrossRef]

62. Hayashi, O.; Katagiri, M.; Rothberg, S. Mechanism of the pyrocatechase reaction. *J. Am. Chem. Soc.* 1955, 77, 5450–5451. [CrossRef]

63. Ozawa, K.; Iwasa, H.; Sasaki, N.; Kinoshita, N.; Hiratsuka, A.; Yokoyama, K. Identification and characterization of thermostable glucose dehydrogenases from thermophilic filamentous fungi. *Appl. Microbiol. BIot.* 2017, 101, 173–183. [CrossRef][PubMed]

64. Deng, J.J.; Huang, W.Q.; Li, Z.W.; Lu, D.L.; Zhang, Y.; Luo, X.C. Biocontrol activity of recombinant aspartic protease from *Trichoderma harzianum* against pathogenic fungi. *Enzyme Microb. Tech.* 2018, 12, 35–42. [CrossRef][PubMed]

65. Gorlatova, N.; Tchorzewski, M.; Kurihara, T.; Soda, K.; Esaki, N. Purification, characterization, and mechanism of a flavin mononucleotide-dependent 2-nitropropane dioxygenase from *Neurospora crassa*. *Appl. Environ. Microb.* 1998, 64, 1029–1033. [CrossRef][PubMed]

66. Francis, K.; Russell, B.; Gadda, G. Involvement of a flavosemiquinone in the enzymatic oxidation of nitroalkanes catalyzed by 2-nitropropane dioxygenase. *J. Biol. Chem.* 2005, 280, 5195–5204. [CrossRef]

67. Thakur, S.; Chakrabarti, A. *Saccharomyces cerevisiae* Hsp30 is necessary for homeostasis of a set of thermal stress response functions. *J. Microbiol. Biotechn.* 2010, 20, 403–409. [CrossRef]

68. Wämelink, M.; Grüning, N.M.; Jansen, E.E.; Blumlein, K.; Lehrcch, H.; Jakobs, C.; Ralsm, R. The difference between rare and exceptionally rare: Molecular characterization of ribose 5-phosphate isomerase deficiency. *J. Mol. Med.* 2010, 88, 931–939. [CrossRef][PubMed]

69. Jauniaux, J.C.; Grenson, M. GAP1, the general amino acid permease gene of *Saccharomyces cerevisiae*. *FEBS J.* 1990, 190, 39–44.

70. Kim, G.J.; Lee, D.E.; Kim, H.S. Construction and evaluation of a novel bifunctional N-carbamylase–D-hydantoinase fusion enzyme. *Appl. Environ. Microb.* 2000, 66, 2133–2138. [CrossRef]

71. Etxebeste, O.; Herrero-Garcia, E.; Cortese, M.S.; Garzia, A.; Oiartzabal-Arano, E.; de los Ríos, V.; Ugalde, U.; Espeso, E.A. GmcA is a putative glucose-methanol-choline oxidoreductase required for the induction of asexual development in *Aspergillus nidulans*. *J. Microbiol. Biotechn.* 2010, 20, 7830–7836. [CrossRef][PubMed]

72. Tomme, P.; Warren, R.A.J.; Gilkes, N.R. Cellulose hydrolysis by bacteria and fungi. *Adv. Microb. Physiol.* 1995, 37, 1–81.

73. Sun, W.; Shen, Y.H.; Yang, W.J.; Cao, Y.F.; Xiang, Z.H.; Zhang, Z. Expansion of the silkworm GMC oxidoreductase genes is associated with immunity. *Insect Biochem. Molec.* 2012, 42, 935–945. [CrossRef][PubMed]

74. Shin, J.Y.; Bui, D.C.; Lee, Y.; Nam, H.; Jung, S.; Fang, M.; Kim, J.C.; Lee, T.; Kim, H.; Choi, G.J.; et al. Functional characterization of cytochrome P450 monooxygenases in the cereal head blight fungus *Fusarium graminearum*. *Appl. Environ. Microb.* 2012, 43, 2053–2067. [CrossRef][PubMed]

75. Johnson, G. The α/β hydrolase fold proteins of mycobacterium tuberculosis, with reference to their contribution to virulence. *Curr. Protein Pept. Sc.* 2017, 18, 190–210. [CrossRef][PubMed]

76. Tisi, A.; Angelini, R.; Cona, A. Wound healing in plants cooperation of copper amine oxidase and flavin-containing polynamine oxidase. *Plant Signal. Behav.* 2008, 3, 204–206. [CrossRef]

77. Piscitelli, A.; Del Vecchio, C.; Faraco, V.; Giardina, P.; Macellaro, G.; Miele, A.; Pezzella, C.; Sannia, G. Fungal laccases: Versatile tools for lignocellulose transformation. *Comptes Rendus Biol.* 2011, 334, 789–794. [CrossRef]

78. Langfelder, J.; Jahn, B.; Gehringer, H.; Schmidt, A.; Wanner, G.; Brakhage, A.A. Identification of a polyketide synthase gene (pksP) of *Aspergillus fumigatus* involved in conidial pigment biosynthesis and virulence. *Med. Microbiol. Immunol.* 1998, 187, 79–89. [CrossRef]

79. Jeon, J.H.; Kim, S.-J.; Lee, H.S.; Cha, S.-S.; Lee, J.H.; Yoon, S.-H.; Koo, B.-S.; Lee, C.-M.; Choi, S.H.; Lee, S.H.; et al. Novel metagenome-derived carboxylesterase that hydrolyzes β-lactam antibiotics. *Appl. Environ. Microb.* 2011, 77, 7830–7836. [CrossRef]