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Teasing apart a three-way symbiosis: Transcriptome analyses of *Curvularia protuberata* in response to viral infection and heat stress

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ABSTRACT

The fungus *Curvularia protuberata* carries a dsRNA virus, Curvularia thermal tolerance virus, and develops a three-way symbiotic relationship with plants to enable their survival in extreme soil temperatures. To learn about the genome of *C. protuberata* and possible mechanisms of heat tolerance a collection of expressed sequence tags (ESTs) were developed from two subtracted cDNA libraries from mycelial cultures grown under control and heat stress conditions. We analyzed 4207 ESTs that were assembled into 1926 unique transcripts. Of the unique transcripts, 1347 (70%) had sequence similarity with GenBank entries using BLASTX while the rest represented unknown proteins with no matches in the databases. The majority of ESTs with known similarities were homologues to fungal genes. The EST collection presents a rich source of heat stress and viral induced genes of a fungal endophyte that is involved in a symbiotic relationship with plants. Expression profile analyses of some candidate genes suggest possible involvement of osmoprotectants such as trehalose, glycine betaine, and taurine in the heat stress response. The fungal pigment melanin, and heat shock proteins also may be involved in the thermotolerance of *C. protuberata* in culture. The results assist in understanding the molecular basis of thermotolerance of the three-way symbiosis. Further studies will confirm or refute the involvement of these pathways in stress tolerance.

1. Introduction

In nature, most plants develop symbiotic relationships with fungal endophytes that provide them with improved abiotic and biotic stress tolerance [1,2]. The fungal endophyte *C. protuberata* is naturally associated with panic grass (*Dichanthelium lanuginosum*) growing in geothermal soils that can reach 65 °C. The symbiotic relationship between the fungus and the plants is responsible for the survival at high temperature and neither partner can survive temperatures above 38 °C in isolation [3]. A third symbiont, a dsRNA mycovirus, Curvularia thermal tolerance virus (CThTV) is also required [4]. When the fungus is cured of CThTV, neither the plant nor the fungus is able to grow at higher temperatures. However, upon fungal re-infection with CThTV, plants colonized with the fungus regain thermotolerance, confirming the involvement of the virus in the three-way mutualistic symbiosis [4]. CThTV-infected *C. protuberata* confers heat tolerance not only to its native monocot host but also to several other hosts including tomato, a eudicot, indicating that the underlying mechanism is conserved between widely divergent groups of plants. The mechanisms of thermotolerance conferred by the three-way symbiosis are unknown.

Mycoviruses are widespread in fungi but very little is known about their biology due to their symptomless characteristics [5]. The association between mycoviruses and disease attenuation was the first indication of the biological importance of mycoviruses. The presence of mycovirus in several fungal pathogens attenuate fungal virulence including the causal agents of chestnut blight [6], Dutch elm disease [7], Victoria blight of oats [8], and *Sclerotinia sclerotiorum* [9]. ESTs are an effective method to understand the role of mycoviruses in their host [10].

Little sequence information is available for the mutualistic fungal endophytes. In this study, we developed a collection of ESTs from two subtracted cDNA libraries generated from *C. protuberata* with or without CThTV. The data provided candidate genes for further studies on the role of the fungal endophyte in the three-way symbiosis, and insights into possible mechanisms of thermotolerance and the role of CThTV.

Abbreviations: CThTV, Curvularia thermal tolerance virus; ESTs, expressed sequence tags; An, *C. protuberata* strain containing CThTV; VF, C. *protuberata* strain without CThTV; VIC-ESTs, viral induced transcripts under control conditions; VIH-ESTs, viral induced transcripts under heat stress conditions.

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2. Materials and methods

2.1. Fungal strains and culture conditions

The *C. protuberata* strains An (containing CThTV) and VF (without CThTV) were described previously [4]. Small and equal sized pieces of the original fungal cultures were used to inoculate the center of a Petri dish containing 0.1X potato dextrose (PD) broth supplemented with ampicillin, kanamycin and streptomycin. Six replicates for each fungal strain and temperature treatment were used. Plates were incubated for six days at 25 °C, 30 °C or 38 °C, then growth was visually assessed, and cultures were photographed. For spore viability, 12 freshly inoculated plates of each strain were grown at 25 °C for five days and then six plates of each were moved to a 38 °C incubator for 24, 48 or 72 h while the rest of the plates were kept at 25 °C. After the heat treatment, five small disks of each fungal plate were cut and resuspended in 1 ml of water containing 1% Tween20. The total number of spores from each disk was counted using a hemocytometer and an average number of spores were calculated. A 200 μl aliquot of each spore suspension was spread over each of five glass slide, the slides were dried, and incubated in high a moisture container at 25 °C for 48 h to allow germination. The number of germinated spores were counted and normalized to the total number of spores of each suspension. Spore viability under heat stress was calculated as relative to the number viable spores at 25 °C.

2.2. Sample preparation for RNA isolation

A starter culture of each fungal strain was grown in a 250 ml flask containing 100 ml of 0.1X PD broth supplemented with antibiotics at 25 °C with shaking for 5 days. The starter cultures were used to inoculate fresh 0.1X PD broth cultures (three replicates of each sample and time point). The fresh cultures of the An and VF fungi were grown at 25 °C for five days with shaking. After the initial growth period, the control samples were kept at 25 °C while the heat-stressed samples were moved to another incubator set at 38 °C. Mycelial cultures were collected after 1, 3, 6, 9, 12 and 24 h using filter papers and vacuum, immediately frozen in liquid nitrogen, lyophilized overnight and then kept at −80 °C until further analyses.

2.3. RNA extraction and subtracted cDNA library construction

Two-hundred mg of each lyophilized mycelia tissue sample were used for RNA extraction using the RNeasy Mini Kit (Qiagene). Equal amounts of total RNA from each biological replicate and time point were pooled prior to isolation of poly(A)$^+$ RNA using the PolyATtract mRNA isolation kit (Promega). Four mRNA samples were generated: (1) An control grown at 25 °C; (2) An heat grown at 38 °C; (3) VF control grown at 25 °C; and (4) VF heat grown at 38 °C. Two μg of each mRNA sample was used for subtraction hybridization, using the Clontech PCR-Select cDNA Subtraction kit (Clontech). Two subtractions were performed to enrich for viral induced transcripts under control conditions (VIC-ESTs) and viral induced transcripts under heat stress conditions (VIH-ESTs). Briefly, in the first subtraction, the poly(A)$^+$ RNA isolated from the An control was used as the "tester" cDNA and samples isolated from the VF control were used as the "driver" or the reference cDNA. This subtraction yielded enrichments of fungal transcripts induced by CThTV infection under normal growth conditions (VIC-ESTs). In the second subtraction, the poly(A)$^+$ RNA isolated from the heat treated An strain was used as the "tester" while the heat treated VF mRNA was used as the "driver" cDNA. This subtraction yielded viral induced ESTs under heat stress (VIH-ESTs). The subtracted PCR products were then cloned into pGEM-T Easy (Promega). From each library, 2880 white bacterial colonies were inoculated into 384-well plates with 200 μl terrific broth media with ampicillin.

2.4. EST sequencing and analysis

Plasmids of cloned cDNA were isolated using the standard alkaline lysis method and sequenced on a 3730 DNA Analyzer (Applied Biosystems) using the M13 reverse primer. Low quality, vector and primer sequences were removed and then assembled by using DNASTAR Lasergene 8 software. The assembled transcripts were annotated based on BLASTX searches against the latest NCBI NR, UniProtKB/Swiss-Prot, UniProtKB/TrEMBL and the GO reference database. All individual EST sequences have been deposited in the NCBI dbEST database under accession numbers 70403454 to 70407660.

2.5. Real-time quantitative RT-PCR (qRT-PCR)

The total RNA used for library constructions was used to validate the EST results using qRT-PCR. Total RNA (2 μg) was converted into cDNA using SuperScript III (Invitrogen). The qPCR was performed using a Power Sybr Green MASTER MIX and an ABI7900HT thermal cycler (Applied Biosystems). Gene expression was measured in triplicate and was calculated by the comparative Ct method (Applied Biosystems). Expression levels were normalized to the 40s rRNA. All primers are listed in Supplementary Table S1.

3. Results and discussion

3.1. Growth and spore viability of C. protuberata under different temperatures

The three-way symbiosis enables the plant, fungus and virus to survive soil temperatures up to 65 °C, however, the fungus cannot grow in culture above 38 °C. We compared the effects of different temperatures on the growth of *C. protuberata* carrying the CThTV (An) and the virus-free fungus (VF) in culture. Both fungal strains had comparable growth at 25 °C, while the VF growth was decreased compared to the An strain when temperature was increased to 30 °C. Both fungal strains showed significant growth reduction at 38 °C (Supplementary Fig. S1a). In addition, exposure of the fungal cultures to 38 °C for more than 24 h reduced spore viability of the VF strain significantly compared to the An strain (statistics using student's *t* test, Supplementary Fig. S1b). These results indicate that under culture conditions the An strain is more thermotolerant than the VF strain.

3.2. Generation of ESTs from C. protuberata cDNA libraries

To detect potentially up-regulated RNAs induced by CThTV and/or heat stress, two subtracted cDNA libraries were constructed followed by single-pass sequencing. Through subtraction, the targeted enrichment of transcripts from these two *C. protuberata* libraries are: (1) VIC-ESTs, up-regulated transcripts due to the presence of the CThTV under control conditions; and (2) VIH-ESTs up-regulated transcripts in response to virus under heat stress conditions. Reverse northern was used as a preliminary means to assess differential expression of generated ESTs. Identical membranes were spotted with subtracted cDNA clones and then hybridized with DIG-labeled cDNA generated from RNA isolated from An and VF strains under control conditions, to compare relative abundance of these clones in the *C. protuberata* with or without the

CThTV. Most of the spotted clones have higher intensity in the An control compared to the VF control, indicating efficient cDNA subtraction (Supplementary Fig. S2). We sequenced 2880 ESTs from each library. After removal of the vector and low quality sequences, a total of 4207 high quality EST sequences were generated. ESTs were assembled into unique transcripts to assess the level of redundancy and to estimate the number of putative genes represented. EST assembly identified 611 contigs and 1315 singletons for a total of 1926 unique transcripts. The majority of contigs (563) were composed of two to ten ESTs, while 48 contigs were composed of 11 to 74 ESTs, thus representing highly expressed genes.

3.3. Functional annotation of ESTs

To assess the function of heat and viral-responsive genes, the entire set of 1926 assembled unique transcripts were annotated based on the top matches with known genes retrieved by BLASTX searches against different databases. We identified 1347 transcripts (70%) with matches to known genes in the databases, while 578 transcripts (30%) had either no similarity to any sequence in the databases, or poor matches. There was one unique transcript assembled from six ESTs representing the sequence of the CThTV. Of the 1347 annotated transcripts with known similarities, 1308 (97%) were most similar to genes in fungal ascomycota (Supplementary Table S2). The ESTs with similarity to known or putative genes were then grouped into major Gene Ontology (GO) categories (http://www.geneontology.org). A majority of ESTs (69%) had no matches with any of the GO categories while the molecular function, cellular component and biological process categories were represented by 1%, 3% and 27% of the ESTs, respectively (Fig. 1A). After normalization, EST distribution across the two libraries was assessed. Of the 1347 unique transcripts that produced significant matches with GenBank entries, 221 transcripts (16.4%), distributed over different functional categories, were common between the two libraries (Supplementary Table S2). An additional 502 transcripts were unique to VIH-ESTs and 624 transcripts were unique to VIC-ESTs (Fig. 1B).

To obtain insight into particular processes activated in response to the presence of CThTV or heat stress, we tentatively classified the 1347 unique transcripts with known matches into 10 different functional classes according to their biochemical function using gene function databases like GO, Uniport (http://www.uniprot.org/downloads), Metacyc (http://www.metacyc.org), COG (http://www.ncbi.nlm.nih.gov/COG) and by a PubMed literature search. Since a protein may be involved in more than one functional category [11], manual classification was necessary in some cases. Using genes unique to each library, we calculated the percentage of normalized ESTs belonging to each of these functional categories (Fig. 2). Functional categorization of both EST groups yielded about 40% of the expressed genes with unknown functions. The percentage of genes belonging to stress response and transport were comparable in both groups of ESTs, however, the percentage of genes in other functional categories were different. To look at any potential direct effects in *C. protuberata* stress tolerance, we analyzed temporal expression profiles of some genes of interest.

3.4. Trehalose

We expected to identify more stress related genes within the VIH-ESTs, but the number of ESTs in this category was similar in both libraries. However, expression profiles of specific genes showed significant differences between the An and VF strains under heat stress conditions. Trehalose biosynthetic pathway gene trehalose phosphatase (*TPS*) was identified in both EST groups. In fungi, trehalose is an important reserve carbohydrate, and plays a role in various environmental stress tolerances as an osmoprotectant that preserves membrane and protein integrity [12–14]. Within the first hour of heat stress, *TPS* showed a two fold increase in the An fungus while the VF expression was down-regulated by at least one fold compared to the control conditions (Fig. 3A). However, between six and nine hours of stress the expression of *TPS* gene was reversed in both fungal genotypes and then reached similar levels after 24 h. Since trehalose is a stable energy-rich molecule found in all organisms except vertebrates [15], controlling the expression of trehalose biosynthetic genes may contribute to regulation of fungal energy sources during stress, in addition to its osmoprotectant properties.

3.5. Glycine betaine

Glycine betaine is a very efficient osmolyte that accumulates in response to different stresses in a wide range of bacterial, fungal and plant species [16]. Identification of several ESTs encoding a homologue of betaine aldehyde dehydrogenase (Badh), the enzyme that catalyzes the second step in the synthesis of glycine betaine, was interesting. The expression profile of *Badh* revealed a four fold increase in the An strain and a two fold increase in the VF strain within the first 3 h of heat stress (Fig. 3B).

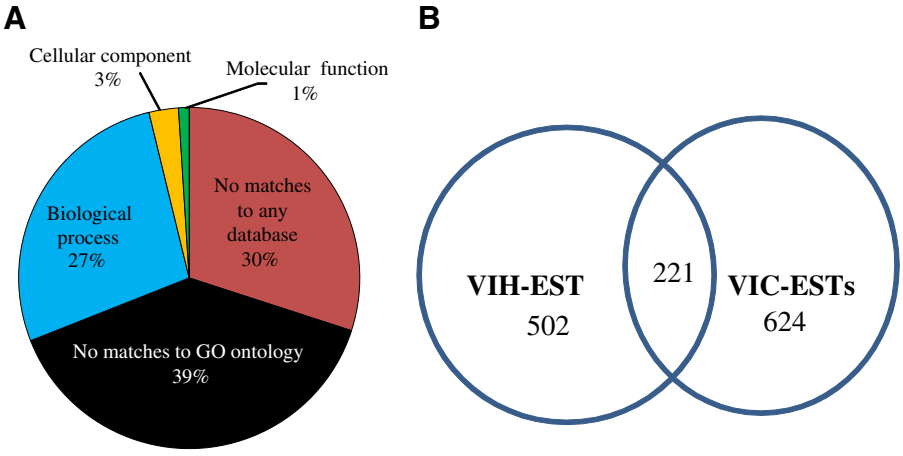

Fig. 1. Functional categories of *C. protuberata* transcripts and exclusive and overlapping sequences between the VIC-ESTs and VIH-ESTs. (A) functional categories of the assembled unique transcripts from the two libraries of *C. protuberata* ESTs; (B) Venn diagram representing unique and common *C. protuberata* transcripts between the two groups of ESTs.

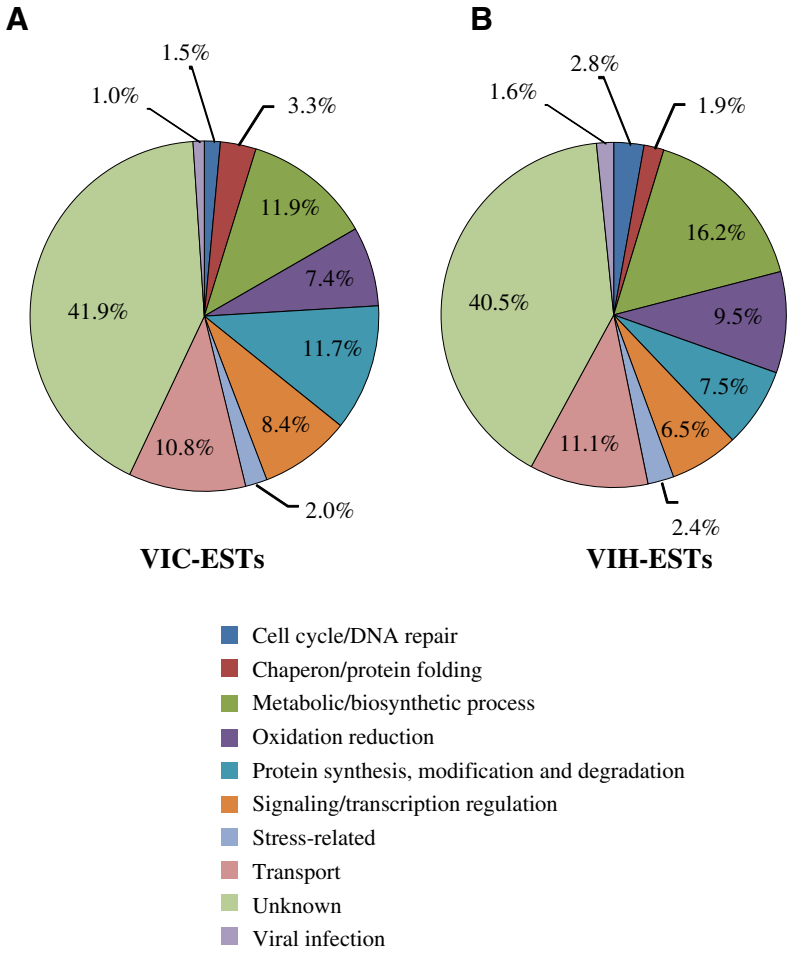

Fig. 2. Summary of gene ontology annotation of VIC-ESTs and VIH-ESTs. (A) viral induced ESTs under control conditions (VIC-ESTs); (B) viral induced ESTs under heat stress (VIH-ESTs).

3.6. Taurine

The most abundant VIH-ESTs encode the enzyme taurine catabolism dioxygenase (*TauD*) with three different orthologs detected. In addition, five orthologs representing the α-ketoglutarate-dependent taurine dioxygenase were identified in the same group of VIH-ESTs. These genes are involved in the catabloic pathway of the β amino acid taurine (2-aminoethanesulfonic acid). In mammalian cells, taurine functions as an osmoprotectant and plays an essential role in antioxidation and detoxification [17,18]. High levels of taurine and hypotuarine were observed in marine invertebrates associated with thiotrophic endosymbionts at hydrothermal vents, and these may play important roles as osmoprotectants in harsh environmental conditions [19,20]. In addition, taurine was found in the desert plants *Opuntia ficus-indica* of the cactus family that grows under heat and drought [21,22] suggesting involvement of taurine in stress tolerance. Hence taurine may function as an osmoprotectant in fungal cells as well. Expression profiling of the *TauD* in *C. protuberata* under control and heat stress conditions showed significant increases in expression in the An compared with the VF strain (Fig. 3C). In microbes, taurine degradation controls nitrogen and sulfur availability and can be used as a carbon source. Therefore, these data may indicate that different levels of taurine degradation between the An and the VF fungi in response to heat stress *via* TauD are used to control carbon utilization.

3.7. Melanin

An abundant EST belonging to metabolic and biosynthetic processes was the scytalone dehydratase (*SCD*). The *SCD* ESTs in the VIH-ESTs library was increased 10-fold over the VIC-ESTs. SCD is a key enzyme in the melanin biosynthetic pathway, and melanin is a predominant pigment in the *Curvularia* genus. In addition to *SCD*, other genes related to the melanin biosynthetic pathway including the genes for malonyl CoA and laccase were found only in the VIH-ESTs. Melanin has been implicated in increased virulence of the human pathogenic fungus *Cryptococcus neoformans* [23]. Melanin is also involved in stress tolerance of rock-inhabiting fungi [24] and in protecting ascomycetes against radiation and extreme temperatures in space craft and nuclear reactors [25,26]. The qPCR of *SCD* showed that fungal tissues carrying CThTV have a 100 fold increase of SCD transcripts within the first hour of heat stress, while VF showed a maximum expression of a 50-fold increase after 3 h of stress (Fig. 3D).

3.8. Heat shock proteins (HSPs)

Viruses use different strategies to recruit host translational machinery including increasing the expression of protein synthesis machinery [27]. In the current study, the number ESTs related to protein synthesis, modification and degradation was higher in the VIC-ESTs compared to the VIH-ESTs, suggesting a role of these

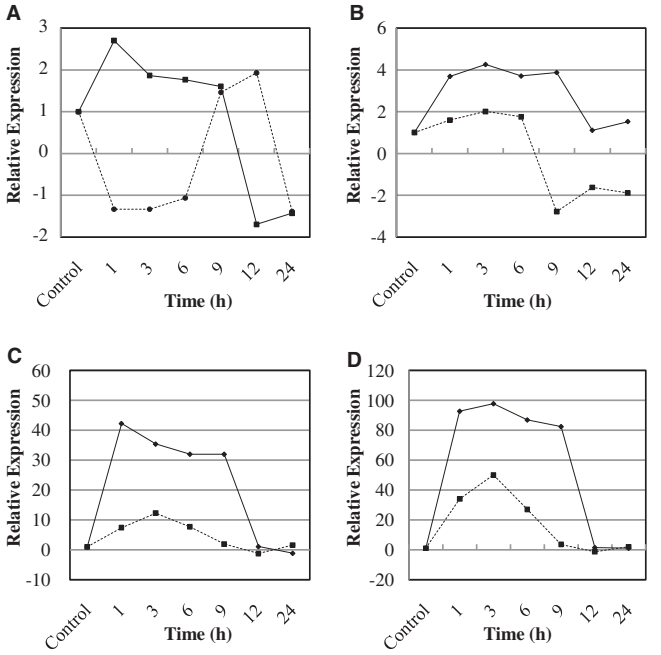

Fig. 3. qRT-PCR analysis of differentially expressed transcripts during heat stress. (A) trehalose phosphatase, (B) betaine aldehyde dehydrogenase, (C) taurine catabolism dioxygenase, (D), and scytalone dehydratase. Solid line: *C. protuberata* An strain; dotted line: *C. protuberata* VF strain.

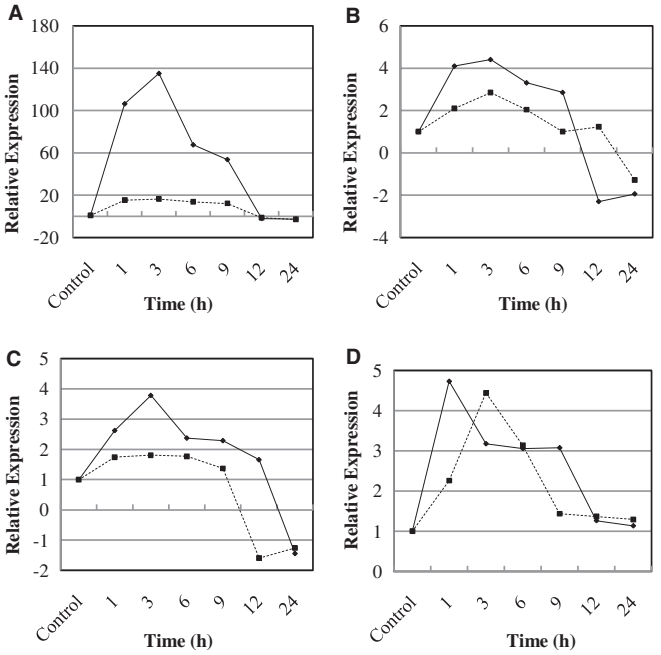

Fig. 4. qRT-PCR analysis of differentially expressed transcripts during heat stress. (A) *HSP30*, (B) glutathione S-transferase III, (C) glutathione S-transferase II, and (D) peroxidase/catalase. Solid line: *C. protuberata* An strain; dotted line: *C. protuberata* VF strain.

processes in maintaining viral replication in the An strain. Several heat shock protein (HSP) transcripts were common between both libraries including *HSP30* and *HSP70*. HSPs are involved in different stress responses, including heat stress in baker's yeast and in several fungal plant pathogens [28,29]. In addition, HSPs are involved in a wide range of other biological processes including oxidative stress, signal transduction, energy production, ubiquitination and proteolysis. Hence expression profiles of *HSP30* and *HSP70* were of particular interest. The *HSP30* transcripts showed significant up-regulation in the An strain under heat conditions, suggesting involvement in heat stress tolerance of the fungus (Fig. 4A). However, the expression profile of *HSP70* was identical between the An and VF strains (data not shown).

3.9. Glutathione S-transferases and catalase

Several transcripts related to reactive oxygen species detoxification enzymes were identified in the VIH-ESTs. These transcripts included five glutathione S-transferase (GST) orthologs and a peroxidase/catalase gene. Expression profiles of GSTII and GSTIII showed increased expression in both fungal cultures under heat stress. However, An showed higher expression of both genes compared to VF during the course of heat treatment until 24 h, after which expression levels in both cultures was minimal (Fig. 4B and c). In *Cryphonectria parasitica* infected with Chryphonectria hypovirus 1, expression of GST was induced to facilitate viral functions [30]. Similarly, we may speculate that the expression pattern of GST genes in the VIH-ESTs is related to CThTV infection and may contribute to the heat stress tolerance by preventing cellular damage in *Curvularia protuberata*. The peroxidase/catalase homologue was present only in the VIC-ESTs. The expression profile of the peroxidase/catalase homologue using qRT-PCR showed a five fold increase within the first hour of heat stress in the An strain while the same level of expression in VF was not reached until three hours. Beyond the first three hours, the expression of the peroxidase/catalase transcripts was similar in both fungal cultures (Fig. 4D). Enzymatic analyses of the catalase in both fungal genotypes showed higher activities in response to heat stress in the An strain (data not shown). Early increase of the peroxidase/catalase expression in An may be crucial in preventing cellular damage during the early stages of stress.

In summary, we identified subsets of *C. protuberata* genes induced by the dsRNA virus, CThTV, under control and heat stress conditions. Functional annotation and expression profiling implicated some genes in heat stress tolerance of the *C. protuberata* carrying CThTV. Genes encoding key enzymes in the biosynthesis and degradation of osmoprotectants including trehalose, glycine betaine and taurine were highly induced in *C. protuberata* carrying CThTV compared to the virus-free fungus in response to heat stress. This observation suggests possible roles for osmoprotectant accumulation and turnover in heat stress tolerance of *C. protuberata*. In addition, significant induction of a gene encoding a key enzyme in the melanin biosynthesis pathway in the An strain suggests a possible role for melanin in the thermotolerance of the fungus contributed by the presence of CThTV. Other candidate genes had differential expression between the An and VF fungi in response to heat stress and also may be contributing to fungal thermotolerance. Further studies on candidate genes will provide insights into the complex thermotolerance of the three-way symbiosis.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2010.09.034.

References

[1] A.E. Arnold, L.C. Mejia, D. Kyllo, E.I. Rojas, Z. Maynard, N. Robbins, E.A. Herre, Fungal endophytes limit pathogen damage in a tropical tree, Proc. Natl. Acad. Sci. USA 100 (2003) 15649–15654.

[2] R.J. Rodriguez, J.F. White Jr, A.E. Arnold, R.S. Redman, Fungal endophyes: diversity and functional roles, New Phytolo. 182 (2009) 314–330.

[3] R.S. Redman, K.B. Sheehan, R.G. Stout, R.J. Rodriguez, J.M. Henson, Thermotholerance generated by plant/fungal symbiosis, Science 298 (2002) 1581.

[4] L.M. Márquez, R.S. Redman, R.J. Rodriguez, M.J. Roossinck, A virus in a fungus in a plant–three way symbiosis required for thermal tolerance, Science 315 (2007) 513–515.

[5] T.E. Cole, B.M. Müller, Y. Hong, C.M. Brasier, K.W. Buck, Complexity of virus-like double-stranded RNA elements in a diseased isolate of the dutch elm desease fungus *Ophiostoma novo-ulmi*, J. Phytopathol. 146 (1998) 593–598.

[6] D.L. Nuss, Hypovirulence. mycoviruses at the fungal-plant interface, Nat. Rev. Microbiol. 3 (2005) 632–642.

[7] K.W. Buck, C.M. Brasier, M. Paoletti, L.J. Crawford, Virus transmission and gene flow between two species of the dutch elm disease fungi, Ophiostoma ulmi and O. novo-ulmi: deleterious viruses as selective agents for gene introgression, in: R.S. Hails, J.E. Beringer, H.C.J. Godfray (Eds.), Genes in the Environment, Blackwell Publishing, 2001, pp. 26–45.

[8] T. Zhao, W.M. Havens, S.A. Ghabrial, Disease phenotype of virus-infected *Helminthosporium victoriae* is independent of overexpression of the cellular alcohol oxidase/RNA-binding protein Hv-p68, Phytopathol. 96 (2006) 326–332.

[9] X. Yu, B. Li, Y. Fu, D. Jiang, S.A. Ghabrial, Y. Peng, J. Xie, J. Cheng, J. Huang, X. Yi, A geminivirus-related DNA mycovirus that confers hypovirulence to a plant pathogenic fungus, Proc. Natl. Acad. Sci. USA 107 (2010) 8387–8392.

[10] J. Shang, X. Wu, X. Lan, Y. Fan, H. Dong, Y. Deng, D.L. Nuss, B. Chen, Large-scale expressed sequence tag analysis for the chestnut blight fungus *Cryphonectria parasitica*, Fungal Genet. and Biol. 45 (2008) 319–327.

[11] M. Ashburner, C.A. Ball, J.A. Blake, D. Botstein, H. Butler, J.M. Cherry, A.P. Davis, K. Dolinski, S.S. Swight, J.T. Eppig, M.A. Harris, D.P. Hill, L. Issel-Tarver, A. Kasarskis, S. Lewis, J.C. Matese, J.E. Richardson, M. Ringwald, G.M. Rubin, G. Sherlock, Gene ontology: tool for the unification of biology, Nat. Genet. 25 (2000) 25–29.

[12] L.K. Conlin, H.C.M. Nelson, The natural osmolyte trehalose is a positive regulator of the heat-induced activity of yeast heat shock transcription factor, Mol. Cell. Biol. 27 (2007) 1505–1515.

[13] T. Hottiger, T. Boller, A. Wiemken, Rapid changes of heat and desiccation tolerance correlated with changes of trehalose content in *Saccharomyces cerevisiae* cells subjected to temperature shifts, FEBS Lett. 220 (1987) 113–115.

[14] M.J. Paul, L.F. Primavesi, D. Jhurreea, Y. Zhang, Trehalose metabolism and signaling, Annu. Rev. Plant Biol. 59 (2008) 417–441.

[15] A.D. Elbein, Y.T. Pan, I. Pastuszak, D. Carroll, New insights on trehalose: a multifunctional molecule, Glycobiology 13 (2003) 17R–27R.

[16] R.A. Muñoz-Clares, Á.G. Díaz-Sánchez, L. Gozález-Segura, C. Montiel, Kinetic and structural features of betwine aldehyde dehydrogenases: mechanistic and regulatory implications, Arch. Biochem. Biophys. 493 (2010) 71–81.

[17] G. Wu, Amino acids: metabolism, functions, and nutrition, Amino Acids 37 (2009) 1–17.

[18] R.J. Huxtable, Physiological actions of taurine, Physiol. Rev. 72 (1992) 101–163.

[19] N. Rosenberg, R.W. Lee, P.H. Yancey, High contents of hypotaurine and thiotaurine in hydrothermal-vent gastropods without thiotrophic endosymbionts, J. Exp. Zoo. 305A (2006) 655–662.

[20] P.H. Yancey, Organic osmolytes as compatible metabolic and counteracting cytoprotectants in high osmolarity and other stresses, J. Exp. Biol. 208 (2005) 2819–2830.

[21] F.C. Stintzing, A. Schieber, R. Carle, Amino acid composition and betaxanthin formation in fruits from *Opuntia ficus-indica*, Planta Med. 65 (1999) 632–635.

[22] F.C. Stintzing, A. Schieber, R. Carle, Phyotchemical and nutritional significance of cactus pear, Eur. Food Res. Technol. 212 (2001) 396–407.

[23] G.Y. Liu, V. Nizet, Color me bad: microbial pigments as virulence factors, Trends in Microbiol. 17 (2009) 406–416.

[24] A.A. Gorbushina, K. Whitehead, T. Dornieden, A. Niesse, A. Schulte, J.I. Hedges, Black fungal colonies as unite of survival: hyphal mycosporines synthesized by rock-dwelling microcolonial fungi, Can. J. Bot. 81 (2003) 131–138.

[25] E. Dadachova, A. Casadevall, Ionizing radiation: how fungi cope adapt, and exploit with the ehlp of melanin, Curr. Opin. Microbiol. 11 (2008) 525–531.

[26] N.V. Mironenko, I.A. Alekhina, N.N. Zhdanova, S.A. Bulat, Intraspecific variation in gamma-radiation resistance and genomci structure in teh filamentous fungus *Alternaria alternata*: a case study of strains inhabiting Chernobyl reactor No.4, Ecotoxicol. Environ. Saf. 45 (2000) 177–187.

[27] M. Bushnell, P. Sarnow, Hijacking the translation apparatus by RNA viruses, J. Cell Biol. 158 (2002) 395–399.

[28] D.L. Jones, J. Petty, D.C. Houle, A. Hayes, S.G. Oliver, I. Riba-Garcia, S.J. Gaskell, L. Stateva, Genome-wide analysis of the effects of heat shock on a *Saccharomyces cerevisiae* mutant with a constitutively activated cAMP-dependent pathway, Comp. Funct. Genom. 5 (2004) 419–431.

[29] D. Albrecht, R. Guthke, A.A. Brakhage, O. Kniemeyer, Integrative analysis of the heat shock response in *Aspergillus fumigatus*, BMC Genomics 11 (2010) 32.

[30] T.D. Allen, A.L. Dawe, D.L. Nuss, Use of cDNA microarrays to monitor transcriptional responses of the chestnut blight fungs *Cryphonectria parasitica* to infection by virulence-attenuating hypoviruses, Eukaryotic Cell 2 (2003) 1253–1265.