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Comparative Transcriptomics of Infectious Spores from the Fungal Pathogen *Histoplasma capsulatum* Reveals a Core Set of Transcripts That Specify Infectious and Pathogenic States

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***Histoplasma capsulatum* is a fungal pathogen that infects both healthy and immunocompromised hosts. In regions where it is endemic, *H. capsulatum* grows in the soil and causes respiratory and systemic disease when inhaled by humans. An interesting aspect of *H. capsulatum* biology is that it adopts specialized developmental programs in response to its environment. In the soil, it grows as filamentous chains of cells (mycelia) that produce asexual spores (conidia). When the soil is disrupted, conidia aerosolize and are inhaled by mammalian hosts. Inside a host, conidia germinate into yeast-form cells that colonize immune cells and cause disease. Despite the ability of conidia to initiate infection and disease, they have not been explored on a molecular level. We developed methods to purify *H. capsulatum* conidia, and we show here that these cells germinate into filaments at room temperature and into yeast-form cells at 37°C. Conidia internalized by macrophages germinate into the yeast form and proliferate within macrophages, ultimately lysing the host cells. Similarly, infection of mice with purified conidia is sufficient to establish infection and yield viable yeast-form cells *in vivo*. To characterize conidia on a molecular level, we performed whole-genome expression profiling of conidia, yeast, and mycelia from two highly divergent *H. capsulatum* strains. In parallel, we used homology and protein domain analysis to manually annotate the predicted genes of both strains. Analyses of the resultant data defined sets of transcripts that reflect the unique molecular states of *H. capsulatum* conidia, yeast, and mycelia.**

Histoplasma capsulatum is a thermally dimorphic fungus that causes the disease histoplasmosis, which is a respiratory or systemic illness that can affect both healthy and immunocompromised individuals. Infections due to *H. capsulatum* are on the rise (1). Although *H. capsulatum* infection can be asymptomatic in healthy hosts, it is estimated that over 50,000 infections cause significant morbidity in immunocompetent individuals each year in the United States alone (2).

H. capsulatum propagates in the soil as an infectious mold that releases asexual spores, known as conidia, into the environment. Conidia are considered the natural infectious particle for *Histoplasma*. The smallest conidia are termed microconidia (ranging from 2 to 6 μm in diameter) and are thought to aerosolize easily and enter pulmonary alveoli due to their small size (3). Once inside the lungs, conidia germinate and give rise to a pathogenic yeast form. Both conidia and the resultant yeast cells are thought to parasitize alveolar macrophages. In contrast, conidia that remain in the environment give rise to filamentous cells after germination. Thus, conidia have the capacity to respond to environmental cues by altering their developmental program.

Very little is known about the biology of *H. capsulatum* conidia, in part because of the difficulty inherent in producing them under laboratory conditions. In previous work, we showed that host macrophages induce different innate immune responses when infected with conidia or yeast cells (4), suggesting that macrophages recognize and respond to an unknown factor(s) that is unique to conidia. Here we describe the development of robust conditions for production and purification of *Histoplasma* conidia, with the goal of characterizing these cells and comparing their transcriptome to those of yeast and mycelia. To identify genes with conserved patterns of expression, we performed these experiments with two highly divergent *H. capsulatum* strains, G217B and G186AR, both of which are commonly studied in the laboratory and have been shown to have diverged evolutionarily by phylogenetic analysis (5). These two strains differ in virulence, cell wall composition, colony morphology, and transformation properties in the laboratory (6, 7). G217B, the more virulent of the two strains, is designated chemotype I and is part of the North American class II (NAm II) clade, characterized by the absence of alpha-1,3 glucan in the cell wall. G186AR is a chemotype II strain and a member of the Panama clade (PAm), which, in contrast to the NAm II clade, is characterized by alpha-1,3 glucan in the cell wall. This carbohydrate polymer contributes to the evasion of immune cell recognition of G186AR (8, 9) but is apparently dispensable for virulence of G217B (10).

Here we used whole-genome oligonucleotide microarrays designed for analysis of the predicted gene set for either the G217B or G186AR strain to compare the expression profiles of the conidia, mycelia, and yeast-form cells of the organism. In parallel, we used BLASTP (11) and protein domain homology results obtained from the NCBI nonredundant (nr) database to manually annotate the entire predicted gene sets from both strains. Whereas large-

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scale analyses of yeast and mycelial enriched transcripts of the G217B strain have been performed previously in our laboratory (12, 13), the data we present herein represent the first analysis of the transcript profile of the infectious conidial form of *H. capsulatum* and the first large-scale analysis of the infectious- and parasitic-phase enriched genes from the G186AR strain. This work defines a core set of conidial, yeast, and mycelial enriched transcripts whose expression pattern is conserved between these two divergent *H. capsulatum* isolates.

MATERIALS AND METHODS

***Histoplasma* strains and media.** *Histoplasma* strains G217B and G186AR in the yeast form were thawed from frozen stocks and routinely cultured on *Histoplasma* macrophage medium (HMM) at 37°C with 5% CO_2 (14). Since we found that prolonged passaging of cultures generally decreased conidial production, cells were passaged no more than 3 times on plates to maintain high levels of conidium production.

Yeast cultures. A fresh plate culture was used to inoculate a 3-day starter culture in Sabouraud dextrose broth (Difco). The starter culture was used to inoculate 50 ml of Sabouraud dextrose broth to a final optical density at 600 nm (OD_{600}) of 0.1. The culture was incubated with shaking at 37°C with 5% CO_2, harvested after 3 days of growth ($OD_{600} = 6$) by vacuum filtration, and snap-frozen with liquid nitrogen.

Mycelial cultures. Yeast cultures were converted to mycelia by plating a heavy inoculum of yeast (from the yeast starter culture described above) onto Sabouraud dextrose agar (Difco) and incubating the plate at 25°C for ~2 weeks. The resulting mycelia were inoculated into Sabouraud dextrose broth and grown for 2 weeks at room temperature with shaking to establish a nonconidiating, vegetative mycelial culture. The shaking mycelial culture was used to inoculate 200 ml of Sabouraud dextrose broth at a 1:50 dilution. One-hundred-milliliter cultures were grown at room temperature with shaking (110 rpm). The mycelia were collected by vacuum filtration, divided into eight 5-ml polypropylene tubes with a cell scraper, and snap-frozen with liquid nitrogen.

Conidial culture and purification. Conidia from the G217B and G186AR strains were obtained by plating approximately 3×10^7 yeast cells on 15-cm petri plates containing soil agar. Soil agar plates were prepared by autoclaving 200 ml soil extract, 1 g yeast extract, 2 g glucose, 15 g Noble agar, and 10 ml 100× Pen/Strep (1× Pen/Strep is 100 U/ml penicillin and 100 μg/ml streptomycin) with the pH adjusted to 6.8. Soil extract was prepared by autoclaving 770 g African violet potting soil with 2 g Na_2CO_3 in 1 liter of water and then filtering the mixture through cheesecloth and Whatman paper. The plates were sealed with Parafilm and stored in plastic tubs at room temperature for 4 to 6 weeks in a biosafety level 3 (BSL3) facility. Conidia were harvested by flooding the plates with phosphate-buffered saline (PBS) and dislodging the conidia with a bent glass rod. Mycelial fragments were removed from the conidial suspension by filtration through sterile glass wool or through 6 to 8 layers of sterile Miracloth (EMD Biosciences). Both microconidia (>95% ± standard deviation [SD]) and macroconidia were routinely obtained from the G217B strain by use of the standard purification protocol. An additional low-speed spin to pellet the large macroconidia improved the final yields of microconidia to >99%. The G186AR strain produced predominantly microconidia (>99% ± SD) from the standard protocol, without the need for additional purification steps. Conidia were pelleted by centrifugation at 2,000 × *g* and 4°C for 10 min, washed in PBS, resuspended in PBS or PBS with 1× Pen/Strep, and stored at 4°C until use. Conidia were enumerated on a hemacytometer following 1:1 dilution in a solution of 37% formaldehyde with a small amount of lactophenol blue. Subsequent dilutions of the conidia were made with PBS. Conidial viability was confirmed by plating serial dilutions on brain heart infusion (BHI) agar with 10% sheep blood, 0.05% cysteine-HCl, and 10 μg/ml gentamicin and incubating them for 10 days or more at 30°C. Twenty to 30% of conidia gave rise to colonies under these conditions. Images of purified spores were obtained with a Zeiss Axiovert 200 inverted microscope using Axiovision 4.4 software and a black-and-white camera. For microscopic studies of germination, spores were transferred into Bird medium (15) supplemented with 84 mg cystine per liter of medium. Germination was assessed visually by quantifying the appearance of yeast or hypha-like protrusions (at 37°C or 27°C, respectively) 48 h after transfer into Bird medium. Images of live cultures of conidia germinating *in vitro* at 27°C and 37°C were taken in a BSL3 facility on a Zeiss Axiovert 200 inverted microscope using Axiovision 4.4 software and a color camera.

Infection of macrophages and mice. Bone marrow-derived macrophages (BMDM) from 8-week-old female C57BL/6 mice were obtained as previously described (4, 16). BMDM were seeded at 2×10^5 cells/well in 24-well dishes in bone marrow-derived macrophage medium and then were incubated overnight at 37°C in 5% CO_2. After 16 to 20 h of growth, macrophages were infected at a multiplicity of infection (MOI) of 10 with conidia that had been washed and resuspended in PBS. Conidia were centrifuged onto macrophages at 50 rpm for 5 min and then incubated at 37°C in 5% CO_2. At the indicated time points, the wells were washed twice in cold PBS and fixed for 5 min in 3.7% formaldehyde diluted in 95% ethanol. The coverslips were then washed two times in double-distilled water (ddH_2O) and stored at 4°C in PBS until ready for staining with periodic acid-Schiff (PAS) base (Sigma-Aldrich) and light methyl green counterstain. Color images of conidia-infected macrophages were obtained on a Zeiss Axiovert 200 inverted microscope using Axiovision 4.4 software and prepared for presentation with Adobe Photoshop.

Mice of the C57BL/6 background (Charles River Laboratories) were anesthetized with isoflurane and infected intranasally with 2×10^6 conidial CFU of the G217B strain in a volume of 25 to 40 μl sterile PBS. At the indicated time points, mice were euthanized using CO_2 inhalation followed by cervical dislocation. Postmortem, the trachea was cannulated and the lungs were inflated *in situ* with 0.7 ml of 10% formalin-PBS. The lungs were then removed and postfixed in 10% formalin-PBS before dehydration in serial alcohols and embedding in paraffin. Five-micrometer parasagittal sections were taken from the right lungs at 100-μm intervals. Sections were stained with hematoxylin and eosin (HE) or PAS and hematoxylin. Sections were analyzed using light microscopy with a Leica DM1000 microscope and were photographed with a Leica DFC290 color camera. All mice were handled according to protocols approved by the UCSF Institutional Animal Care and Use Committee.

RNA preparation. Frozen conidial pellets were homogenized by bead beating with 0.5-mm zirconia-silica beads (Biospec Products, OK) and TRIzol (Invitrogen) in 2-ml screw-cap tubes. RNA was isolated according to the manufacturer's instructions. On average, 3 μg conidial RNA was obtained from each 15-cm plate. Yeast and mycelial RNAs were prepared as previously described (12), with the following modification: pellets were homogenized by bead beating with 0.5-mm zirconia-silica beads in guanidinium lysis buffer. RNA quality was measured with an Agilent Bioanalyzer using RNA Nano LabChips.

Microarray analysis. One microgram of total RNA per conidial, mycelial, or yeast sample was amplified according to the manufacturer's instructions, using a MessageAmp II kit (Ambion). The average yield was 75 μg amplified cRNA (aRNA). Four to five micrograms of aRNA was fragmented with RNA fragmentation reagent (Ambion) prior to hybridizing the samples to microarrays. Samples were labeled with Cy5 or Cy3 and competitively hybridized to custom glass slide 70-mer oligomer microarrays in a closed-circuit experimental design (e.g., direct, pairwise comparisons of yeast and mycelial samples, mycelial and conidial samples, and yeast and conidial samples). Dye-swap hybridizations were performed for these pairwise comparisons. The whole-genome oligonucleotide microarrays representing the gene models predicted by Washington University, St. Louis, MO, were printed at the UCSF Center for Advanced Technology. The microarrays were scanned using Gene PixPro 6.0 software on an Axon 4000B scanner (Molecular Devices). The microarrays were gridded with Gene Pix 6.0 (Molecular Devices) and uploaded to the NOMAD database (http://ucsf-nomad.sourceforge.net/) for quality control, normalization, and data storage. To eliminate the analysis of microarray fea-

tures with low signal intensities, we excluded features from the analysis for which the sum of the medians for the 635-nm and 532-nm channels was ≤500 intensity units. These data were transformed into relative expression levels and analyzed for statistical significance with Bayesian Analysis of Gene Expression Levels (BAGEL) software (17). In the case of G217B, biological replicates were compared to ensure that data were consistent between samples, and representative data were subjected to BAGEL analysis. In the case of G186AR, data from one set of hybridizations were subjected to BAGEL analysis. The data were organized for presentation with Cluster 3 (http://bonsai.hgc.jp/~mdehoon/software/cluster/) and Java Treeview 1.1.4r5 (http://sourceforge.net/projects/jtreeview/).

Conserved yeast, mycelial, and conidial enriched gene sets were defined as orthologous gene pairs with at least 3-fold enrichment in the phase of interest relative to both other phases in both strains. Genes that passed the analogous criterion in only one of the two strains were considered strain specific in their enrichment. For genes probed with two probes, the gene was considered enriched if either probe passed the enrichment criterion.

Northern blots. For Northern blot hybridization, 1 μg of DNase I-treated RNA was loaded onto a MOPS (morpholinepropanesulfonic acid) gel, transferred to nitrocellulose membranes, and subjected to hybridization with a radiolabeled probe as previously described (12). Northern blot probes were generated by PCR with the following primers: CATA-ex3FW3, 5′-TGAAGCCGGAACCTCATAAC-3′; and CATA-ex3RV3, 5′-ATCGTAACCATCCCCAATCA-3′.

Expression constructs. A sequence containing 1,050 bp of the G217B *TYR1* promoter was amplified by PCR using the primer pair OAS1040 (5′-agcacggcgccGATATCTTGTTTGCAGGAAGCCG-3′; NarI site shown in lowercase) and OAS1041 (5′-acggcgtcgacGGGTGACGATATG AAGTTGAGG-3′; SalI site shown in lowercase), cloned into pCR2.1, and sequenced. Positive clones were digested with NarI and SalI and cloned into the NarI-SalI sites of a promoterless green fluorescent protein (GFP)-containing entry vector (Invitrogen) containing the hygromycin resistance gene. Entry vectors were recombined in an LR recombination reaction with the *H. capsulatum* episomal vector pMA35B to generate P_{TYR1}-GFP followed by the terminator of the *CATB* gene (P_{TYR1}-GFP-*CATBt*). P_{CBP1}-GFP-*CATBt* was generated by cloning GFP into the SalI-NotI sites of a P_{CBP1}(p)-*CATBt* entry vector, pLH125 (which contains 1,111 bp of the G186AR *CBP1* promoter), followed by recombination with pMA35B. Expression vectors were digested with PacI and transformed into both G217B and G186AR as previously described (16), using hygromycin resistance as a selectable marker. The resultant yeast-phase transformants were grown in HMM medium at 37°C with 5% CO_2 as described above. To observe GFP expression in mycelia, yeast cultures were inoculated into HMM, aerated at room temperature at 110 rpm, and observed 2 days later. Data are shown only for the G186AR strain (see Fig. 5).

Ortholog prediction and annotation of the *H. capsulatum* gene sets. Orthologous genes between the G186AR and G217B strains as well as between the G217B strain and *Aspergillus nidulans*, *Aspergillus fumigatus*, *Candida albicans*, *Candida glabrata*, and *Saccharomyces cerevisiae* were determined by InParanoid, version 1.35, software, with no outgroup, to determine the best reciprocal BLAST hits between *H. capsulatum* and these species (18). To manually annotate the *Histoplasma* predicted gene sets, BLASTP analysis was performed with the predicted gene models, identified at Washington University, St. Louis, MO, against the NCBI nr database (http://www.ncbi.nlm.nih.gov/GenBank/index.html) between April 2011 and August 2011. These manual gene annotations were further refined using InterProScan protein domain hits (19–21). tRNAs for the G217B strain were predicted by tRNAScan-SE (22). Descriptions for orthologous genes provided in Tables S1 and S2 in the supplemental material were obtained from the *Aspergillus* Genome Database (AspGD) (23; http://www.aspergillusgenome.org/download/chromosomal_feature_files/) for *A. nidulans* and *A. fumigatus*, and gene descriptions for *S. cerevisiae* orthologs were downloaded from the *Saccharomyces* Genome Database (24; http://www.yeastgenome.org/download-data/curation). The annotations from this paper will be made available through HistoBase (http://histo.ucsf.edu/), which will also serve as a tracker for revisions and corrections to the annotation set.

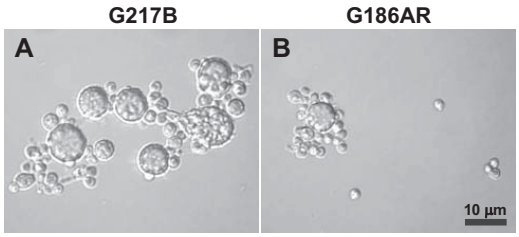

FIG 1 Purified microconidia and macroconidia were generated from *H. capsulatum*. Purified macroconidia and microconidia of the G217B (A) and G186AR (B) strains are shown.

Microarray data accession number. Microarray data are available at the NCBI Gene Expression Omnibus (GEO) under accession number GSE45432.

RESULTS AND DISCUSSION

Purified conidia are viable and infectious. *Histoplasma* mycelia produce two types of spores: microconidia (ranging in size from 2 to 6 μm in diameter) and macroconidia (8 to 14 μm in diameter). The microconidia are thought to be the primary infectious particles due to their small size and potential to enter the small alveoli in the lungs of a host. We found that the strains used in this study made both microconidia and macroconidia (Fig. 1). The conidia produced by the G217B strain were brownish, whereas the conidia produced by the G186AR strain were white or colorless. Purification of *H. capsulatum* conidia typically yielded 95% or more microconidia from the G217B strain, whereas the G186AR strain produced >99% microconidia (data not shown). Conidial viability was routinely assessed by plating for CFU (see Materials and Methods).

In the laboratory, temperature is a sufficient signal to trigger growth in either the filamentous form (at room temperature) or the yeast form (at 37°C) (25), though this temperature-dependent developmental regulation has largely been observed with vegetatively growing cultures. To determine whether conidial germination yields filaments or yeast-phase cells in a temperature-dependent manner, we performed a time course of germination at 27°C and 37°C (Fig. 2) by placing purified conidia in fresh medium. We observed that germination in liquid culture was asynchronous and that, over the course of the experiment, some conidia failed to germinate irrespective of temperature. Those that did germinate displayed initial morphological changes at approximately 24 h postinoculation at 27°C and 37°C. Visual inspection revealed that 48 h after transfer to germination medium at 27°C, 71% of conidia ($n = 78$) displayed hyphal projections or protuberances. Similarly, 86% of conidia ($n = 237$) gave rise to one or more yeast cells by 48 h after transfer to germination medium at 37°C. Hence, the majority of conidia that germinated at 27°C generated filamentous cells, whereas the majority of the conidia germinated at 37°C generated yeast-phase cells, indicating that conidial differentiation was temperature responsive.

To determine whether purified conidia germinated into the yeast form within macrophages, we infected murine BMDM with conidia and monitored fungal cellular morphology over time. At early time points, the conidia readily bound to and

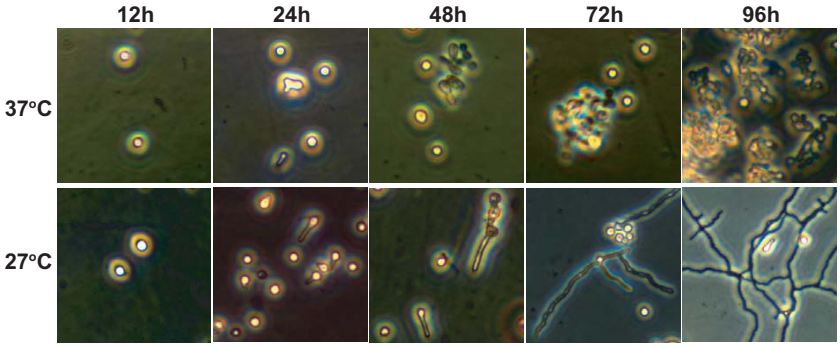

FIG 2 Conidia germinate *in vitro* into filaments at 27°C and into yeast-phase cells at 37°C. A time course of germination of G217B conidia was performed at the indicated temperatures.

were phagocytosed by the macrophages (Fig. 3A and B). Germination of conidia into the yeast form within macrophages was observed by 24 h postinfection (hpi) (Fig. 3C). By 72 hpi (Fig. 3D), the resultant yeast cells had proliferated robustly within macrophages, ultimately causing host cell lysis. Occasionally, conidia also produced mycelial-form cells within macrophages incubated at 37°C (Fig. 3E).

To examine the fate of purified conidia in the context of an infection, we infected mice intranasally with G217B conidia and monitored fungal cell morphology and dissemination over time. Although the only cell type present in the inoculum was conidia, by 5 days postinfection (dpi) both conidia and yeast were observed in the lung (Fig. 3F), strongly suggesting that yeast cells arose from germinating conidia. Since multiple yeast cells were observed in close association with conidia at day 5 (Fig. 3F), it is possible that at least some conidia are capable of continued production of yeast cells in the lungs. Interestingly, histological analysis showed that conidia continued to persist in the lung at later time points, indicating that conidial cells are resistant to destruction by the host. Spleens from conidia-infected mice ultimately became colonized with yeast cells, as we observed previously (4), indicating that conidia from a purified laboratory strain are capable of causing disseminated disease in a mouse model of pulmonary histoplasmosis. The conidia themselves were never observed in the spleens of infected mice over a range of time points (data not shown), suggesting that conidial cells cannot transit from the lung and that germination into the yeast form is required for dissemination to the spleen.

Identification of differentially expressed transcripts in two divergent *H. capsulatum* strains. Once we established that purified conidia were viable and infectious, we wanted to determine which transcripts showed enrichment in conidia versus yeast-form or mycelial cells. We reasoned that we could identify phase-specific enriched transcripts that showed conserved patterns of expression by comparing the transcriptomes of conidia, yeast, and mycelia from two divergent *H. capsulatum* strains, i.e., G217B and G186AR. In parallel with these studies, we also manually annotated the gene predictions from each genome, as shown in Table S1 (G217B) and Table S2 (G186AR) in the supplemental material. We utilized two approaches to annotate the *Histoplasma* predicted gene sets. The first was analysis and manual annotation of the predicted genes based on BLASTP homology to protein matches at the NCBI nr database. The second was the assignment of Gene Ontology (GO) annotations for orthologs of characterized proteins of *Saccharomyces cerevisiae*, *Aspergillus nidulans*, *A. fumigatus*, *Candida albicans*, and *C. glabrata*. These orthology-based computational annotations were examined manually and used to refine the gene descriptions.

To determine which transcripts were enriched in *H. capsulatum* conidia, mycelia, and yeast, we compared the gene expression profiles of purified conidia with those of yeast-form and mycelial cells of both the G217B and G186AR strains by using the BAGEL algorithm (17). This algorithm transforms replicate measurements of expression ratios into relative estimates of per-state expression, where the state of least expression is assigned an expression value of 1.0. Additionally, BAGEL estimates confidence

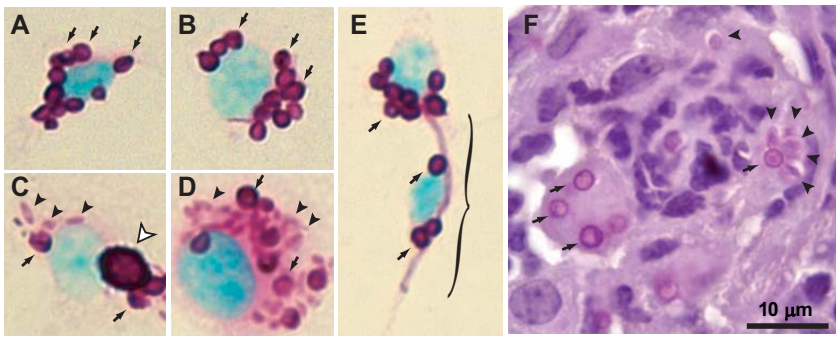

FIG 3 Germination of conidia in macrophages and host tissues. PAS-stained images of conidia-infected bone marrow-derived macrophages are shown at 2 hpi (A), 6 hpi (B), 24 hpi (C), and 72 hpi (D). (E) Occasional mycelial forms observed at 24 hpi. (F) A lung section from a conidia-infected mouse was stained with PAS, hematoxylin, and eosin. Arrows indicate conidia, black arrowheads indicate yeast cells, and the white arrowhead indicates a macroconidium.

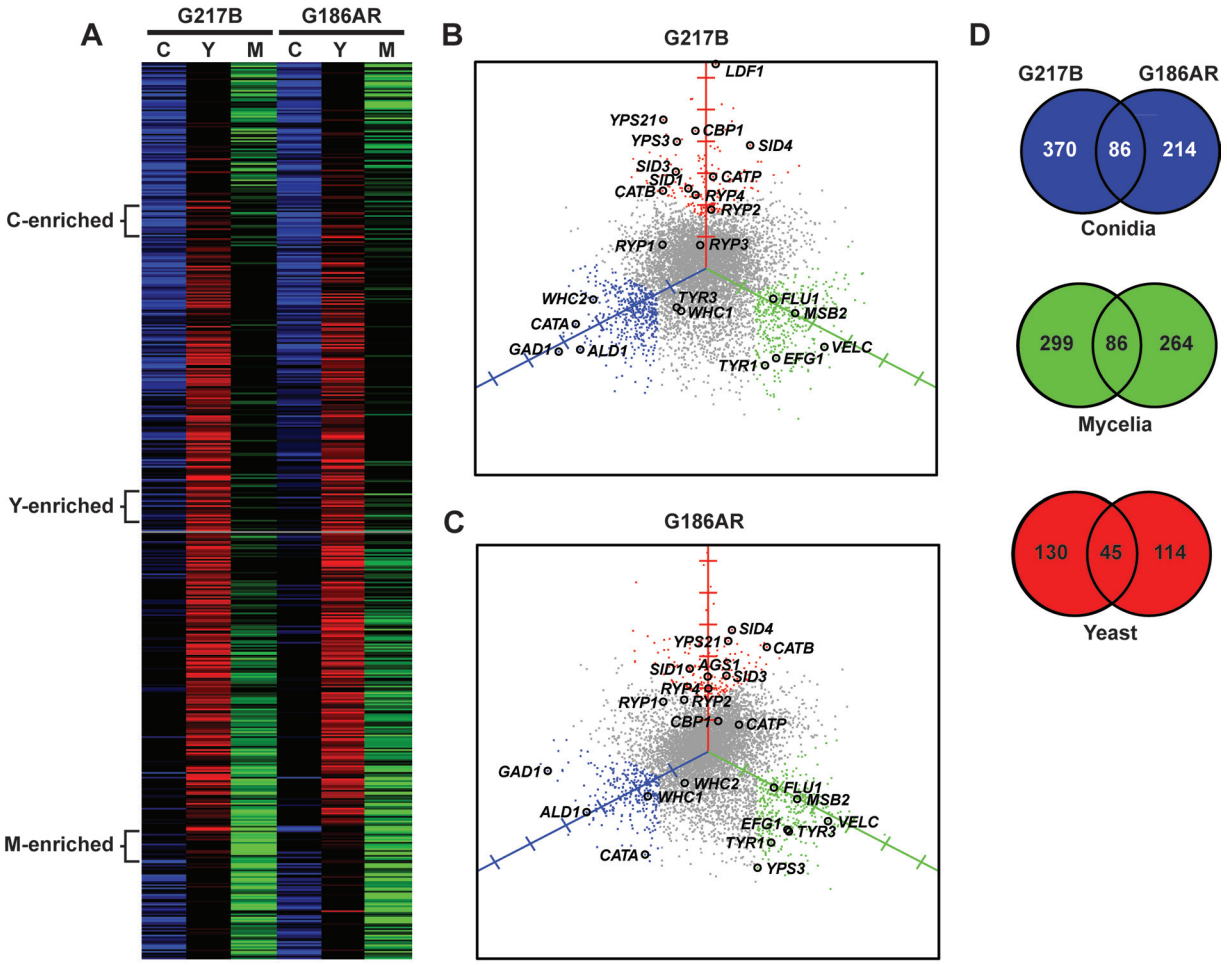

FIG 4 Relative expression of G217B and G186AR transcripts in yeast, mycelia, and conidia. (A) Heat map of relative conidial (C), yeast (Y), and mycelial (M) expression levels for pairs of orthologous genes with data present in both strains. Intensities are $\log_2$ BAGEL-estimated relative expression levels from 0 (black) to 3 (saturated). Genes are ordered by phase specificity, defined as the average angular coordinate from plots B and C. Phase-specific genes are indicated by the bracketed regions to the left of the heat map. Relative enrichment plots are shown for G217B (B) and G186AR (C). Yeast, mycelial, and conidial axes are drawn in red, green, and blue, respectively; axis ticks indicate $\log_2$ units of enrichment. Genes were plotted by projecting the BAGEL-estimated relative expression values on the corresponding axes (the condition of lowest expression always has a $\log_2$ enrichment of zero). Yeast, mycelial, and conidial enriched genes, based on a 3-fold enrichment criterion, are colored red, green, and blue, respectively. Genes of interest mentioned in the text and/or tables are highlighted with black circles and labeled. (D) Venn diagrams showing conserved expression of the 3-fold differentially expressed conidial-, mycelial-, and yeast-specific transcripts of the G217B and G186AR strains.

intervals and probabilities of differential expression between states by sampling possible fits of the data in a Bayesian framework. The relative gene expression profiles of orthologous G217B and G1686AR genes (filtered for data present from the BAGEL statistical analysis of both strains [see Materials and Methods]) in the three phases (conidia, yeast, and mycelia) are shown as a heat map where relative conidial expression is highlighted in blue, relative mycelial expression is in green, and relative yeast expression is in red (Fig. 4A). Despite the evolutionary divergence of G217B and G186AR, we noted a remarkable conservation of phase-specific enriched expression between orthologs of the two strains. To visually illustrate the relative expression of a given transcript in the three phases, Fig. 4B and C show 3-axis plots of the $\log_2$ relative expression values for each gene in the G217B and G186AR data sets, respectively. Specific genes of interest, described below or mentioned in Tables 1 and 2, are highlighted on the plots.

According to the BAGEL analysis, a total of 3,404 genes were differentially expressed in one of the three cell types of G217B, with P values of $\geq$0.985 (see Table S3 in the supplemental material): 1,275 were enriched in conidia, 1,006 were enriched in mycelia, and 1,123 were enriched in yeast. In the G186AR strain, 3,179 genes were differentially regulated by the same criteria (see Table S4): 1,183 were enriched in conidia, 925 were enriched in mycelia, and 1,071 were enriched in yeast. Table S5 reports gene expression data across the three phases for both strains in a gene-centric manner, providing ortholog information across both strains. To define the set of most highly enriched transcripts in conidia in each strain, the results of the BAGEL analysis were filtered with a 3-fold cutoff, such that conidial enriched genes were at least 3-fold enriched relative to those in both yeast and mycelia. Yeast and mycelial enriched genes were defined analogously. Using this stringent 3-fold cutoff, we determined the numbers of strain-specific and conserved phase-specific enriched genes for each strain (Fig. 4D). By these criteria, we identified 456 and 300

TABLE 1 Conserved differentially expressed transcripts of strains G186AR and G217B

Systematic names (G217B and G186AR)[a]	Gene name	Annotation	Fold enrichment relative to lowest expression state[b]					
			G217B			G186AR		
			C	Y	M	C	Y	M
Genes enriched in conidia								
HISTO_DM.Contig933.Fgenesh_histo.44.final_new and Contig1.59.Fgenesh_histo.10.final	*GAD1*	Glutamate decarboxylase (metabolism)	32.3	1.0	1.2	37.1	4.0	1.0
HISTO_ZL.Contig1131.Fgenesh_histo.33.final_new and Contig10.32.eannot.1129.final	*NIT36*	HHE domain-containing protein (nitric oxide response—nitrosative stress response)	27.4	2.7	1.0	29.2	5.5	1.0
HISTO_ZL.Contig658-snap.32.final_new and Contig8.2-snap.39.final	*MBP1*	Macrophage-binding protein, putative (host interaction)	29.5	1.0	3.9	16.4	1.0	1.5
HISTO_ZT.Contig1128f.eannot.1117.final_new and Contig6.17.Fgenesh_Aspergillus.5.final	*ERY1*	Erythromycin esterase/L-isoaspartate *O*-methyltransferase (drug resistance)	22.9	1.0	2.1	19.8	1.5	1.0
HISTO_ZT.Contig181.genewise.118.final_new and Contig1.29.genewise.5.final		NAD-binding oxidoreductase (metabolism)	16.8	2.5	1.0	26.6	3.5	1.0
HISTO_FE.Contig19.eannot.1833.final_new and Contig0.56.eannot.1054.final		Integral membrane protein, possibly mitochondrial (unknown function)	11.7	3.3	1.0	32.3	7.3	1.0
HISTO_ZZ.Contig127b.Fgenesh_histo.85.final_new and Contig9.19.eannot.1051.final	*ALD1*	Aldehyde reductase (metabolism)	24.2	1.0	1.4	15.4	1.1	1.0
HISTO_ZT.Contig174.eannot.1384.final_new and Contig1.53.eannot.1035.final	*CATA*	Catalase A (stress response)	18.9	1.3	1.0	19.0	1.0	4.6
HISTO_ZZ.Contig127b-snap.107.final_new and Contig9.30.eannot.1097.final		Alcohol dehydrogenase (alcohol metabolism)	40.7	1.0	5.2	8.1	1.0	1.5
HISTO_ZZ.Contig127c.Fgenesh_histo.128.final_new and Contig18.4.Fgenesh_histo.51.final	*NIT9*	C2 domain-containing protein (nitrosative stress response)	19.2	1.2	1.0	14.7	1.2	1.0
HISTO_ZZ.Contig127a.fgenesh_plus.7.final_new and Contig9.12.fgenesh_plus.5.final	*CDI1*	Catechol 1,2-dioxygenase (metabolism)	14.2	2.8	1.0	17.4	4.3	1.0
HISTO_GY.Contig471.Fgenesh_histo.44.final_new and Contig5.26.Fgenesh_histo.6.final		BTB domain-containing protein (protein-protein interactions)	25.0	1.2	1.0	9.8	1.0	1.1
HISTO_ZT.Contig1089.genewise.10.final_new and Contig1.43.genewise.59.final		Hemerythrin HHE cation binding domain-containing protein (nitric oxide response)	19.7	1.0	1.3	12.2	2.1	1.0
HISTO_LG.Contig392.eannot.1653.final_new and Contig25.5.eannot.1058.final		Isochorismatase family hydrolase (hydrolysis)	26.8	1.0	4.0	7.6	1.0	1.1
HISTO_FX.Contig167.eannot.1335.final_new and Contig27.4.eannot.1064.final	*OPS1*	Opsin 1, G-protein-coupled receptor (cell signaling)	14.4	3.1	1.0	10.4	1.6	1.0
HISTO_GI.Contig363.eannot.1180.final_new and Contig2.60.Fgenesh_Aspergillus.5.final		LEA domain-containing protein, 9 DUF3659 domains (unknown function)	13.6	1.0	1.9	10.5	1.0	1.2
HISTO_ZL.Contig658.eannot.1262.final_new and Contig8.2.Fgenesh_histo.46.final	*XFP1*	Xylulose-5-phosphate phosphoketolase (metabolism)	12.4	1.0	1.2	10.9	1.0	1.2
HISTO_HS.Contig68.Fgenesh_histo.212.final_new and Contig3.54.eannot.1104.final	*RDS1*	Stress response protein (stress response)	17.6	1.0	1.2	7.5	1.2	1.0
HISTO_DM.Contig936.eannot.1264.final_new and Contig3.11.eannot.1132.final		TshiJ/PfpI family protein, possible chaperone and cysteine protease	7.7	1.0	1.3	14.7	1.0	4.0
HISTO_ZZ.Contig127c-snap.92.final_new and Contig13.16-snap.1.final		Lanthionine synthetase C family protein (metabolism)	9.8	1.0	2.3	11.2	1.0	1.1

HISTO_GI.Contig382.Fgenesh_histo.57.final_new and Contig2.67.eannot.1129.final	*MFS11*	MFS multidrug transporter (drug resistance)	6.9	1.6	1.0	14.9	1.0	1.9
HISTO_ZU.Contig171-snap.5.final_new and Contig31.3-snap.18.final		HhH-GPD domain-containing protein, DUF488 domain	12.0	2.4	1.0	8.3	1.5	1.0
HISTO_HS.Contig68-snap.98.final_new and Contig3.65.Fgenesh_histo.45.final		Glyoxal oxidase (lignin degradation)	11.1	1.0	1.3	7.8	1.5	1.0
HISTO_DA.Contig93.Fgenesh_histo.10.final_new and Contig0.103.Fgenesh_histo.10.final		F-box domain-containing protein, putative (unknown function)	12.2	3.6	1.0	6.8	1.2	1.0
HISTO_LG.Contig392.fgenesh_plus.37.final_new and Contig25.9.fgenesh_plus.2.final		Glutathione *S*-transferase family protein (metabolism)	5.6	1.0	1.2	12.1	1.0	1.7
HISTO_GI.Contig382.Fgenesh_histo.91.final_new and Contig2.67.eannot.1136.final	*PSP1*	Parasitic-phase-specific protein of *Coccidioides* (unknown function)	6.4	1.0	2.1	10.5	1.0	3.0
HISTO_AL.Contig317.eannot.1531.final_new and Contig8.21.Fgenesh_histo.12.final	*TPS3*	Alpha,alpha-trehalose phosphate synthase subunit (trehalose metabolism)	5.2	1.3	1.0	11.4	1.0	1.2
HISTO_ZL.Contig658.eannot.1243.final_new and Contig8.2.eannot.1201.final	*AKI1*	Acetate kinase (metabolism)	10.2	1.1	1.0	5.2	1.0	1.0
HISTO_JG.Contig207.genewise.57.final_new and Contig6.25.Fgenesh_histo.22.final	*ISH1*	Stress response protein (stress response)	8.4	1.2	1.0	6.3	1.0	1.3
HISTO_ZY.Contig518.Fgenesh_histo.22.final_new and Contig5.7.eannot.1127.final		Zinc-binding oxidoreductase, ToxD-like (metabolism)	5.9	1.2	1.0	8.5	1.0	2.3
HISTO_EA.Contig33.genewise.94.final_new and Contig12.16.Fgenesh_Aspergillus.36.final		Transcription factor IIIc-like protein, putative (PolIII transcription)	7.4	1.0	1.0	6.4	1.1	1.0
HISTO_DU.Contig190.Fgenesh_histo.12.final_new and Contig13.7.Fgenesh_Aspergillus.15.final		Carboxypeptidase (proteolysis)	5.7	1.1	1.0	7.8	2.2	1.0
HISTO_ZY.Contig571f.Fgenesh_histo.26.final_new and Contig5.14.Fgenesh_histo.6.final		F-box domain-containing protein, putative (unknown function)	4.3	1.0	1.0	10.0	1.3	1.0
HISTO_ZL.Contig1131.eannot.2111.final_new and Contig10.33.eannot.1051.final		NmrA family transcriptional regulator (transcription)	5.8	1.8	1.0	7.3	1.0	2.2
HISTO_ZL.Contig1161b.Fgenesh_histo.87.final_new and Contig0.33.Fgenesh_histo.6.final		GAL4-like Zn2Cys6 transcription factor (transcription)	6.6	1.0	1.1	5.7	1.0	1.2
HISTO_GB.Contig114.eannot.1124.final_new and Contig26.4.Fgenesh_histo.3.final		Similar to YT521-B-like splicing factor (RNA metabolism)	6.3	1.0	1.2	5.8	1.1	1.0
HISTO_FE.Contig19.Fgenesh_Aspergillus.214.final_new and Contig0.57.Fgenesh_Aspergillus.19.final	*FBD1*	MYB family conidiophore development protein FlbD (asexual development)	5.7	1.0	1.5	6.1	1.4	1.0
HISTO_LF.Contig359.eannot.2006.final_new and Contig2.51.eannot.1195.final	*DNL4*	NHEJ DNA ligase (DNA repair)	7.7	1.9	1.0	4.1	1.0	1.0
HISTO_EA.Contig33.fgenesh_plus.53.final_new and Contig12.9.genewise.4.final	*FET3*	Iron transport multicopper oxidase (iron transport)	6.2	1.8	1.0	5.0	1.5	1.0
HISTO_HS.Contig68.eannot.2168.final_new and Contig3.54.eannot.1111.final		Serine protein kinase (protein phosphorylation)	4.2	1.1	1.0	5.1	1.7	1.0
HISTO_ZT.Contig181.eannot.1629.final_new and Contig1.12.eannot.1068.final		CBS domain-containing protein (unknown function)	4.1	1.0	1.2	5.0	1.0	1.6
HISTO_GY.Contig471.Fgenesh_Neurospora.27.final_new and Contig5.26.eannot.1025.final	*FURA*	Uracil permease (transport)	4.9	1.0	1.4	4.0	1.1	1.0
HISTO_LG.Contig392.genewise.34.final_new and Contig25.9.fgenesh_plus.10.final	*TGL1*	Triglyceride lipase-cholesterol esterase (lipid metabolism)	4.6	1.0	1.3	3.9	1.0	1.0
HISTO_DA.Contig93-snap.60.final_new and Contig0.89-snap.22.final		Alpha/beta hydrolase (hydrolysis)	3.9	1.1	1.0	3.9	1.0	1.0

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TABLE 1 (Continued)

Systematic names (G217B and G186AR)[a]	Gene name	Annotation	Fold enrichment relative to lowest expression state[b]					
			G217B			G186AR		
			C	Y	M	C	Y	M
Genes enriched in yeast								
HISTO_AL.Contig317.eannot.1542.final_new and Contig8.3.eannot.1103.final		Highly yeast-phase-specific transcript (unknown function)	1.4	47.3	1.0	5.0	90.2	1.0
HISTO_GY.Contig460.eannot.1679.final_new and Contig37.5.eannot.1055.final		Highly yeast-phase-specific transcript (unknown function)	1.0	44.8	1.9	1.0	77.3	1.0
HISTO_ZL.Contig1131.eannot.1938.final_new and Contig20.3.eannot.1101.final	*YPS21*	Yeast-phase-specific protein (unknown function)	2.6	41.5	1.0	1.0	14.0	1.6
HISTO_EA.Contig33.eannot.1524.final_new and Contig12.3.eannot.1252.final	*MET16*	3′-Phosphoadenosine-5′-phosphosulfate reductase (metabolism)	5.2	24.8	1.0	2.4	18.1	1.0
HISTO_JG.Contig206.fgenesh_plus.57.final_new and Contig24.6.eannot.1053.final	*SID4*	Acid coenzyme A (coA) ligase (siderophore biosynthesis)	1.0	24.1	2.7	1.0	18.6	1.7
HISTO_ZT.Contig1089.eannot.1639.final_new and Contig1.44.Fgenesh_Aspergillus.6.final	*GNT1*	α*N*-Acetylglucosamine transferase	2.0	16.7	1.0	3.1	15.2	1.0
HISTO_KF.Contig597.eannot.1481.final_new and Contig11.25.eannot.1100.final	*CCP2*	Cytochrome *c* peroxidase (metabolism)	4.6	18.6	1.0	1.0	11.9	1.4
HISTO_JG.Contig206.eannot.1534.final_new and Contig24.6.eannot.1061.final	*OXR1*	Oxidoreductase (siderophore biosynthesis)	1.0	11.7	2.5	1.0	17.2	5.0
HISTO_BP.Contig459-snap.79.final_new and Contig7.33.eannot.1033.final	*CYN1*	High-affinity cystine transporter (transport)	1.0	25.8	1.6	1.0	7.7	1.7
HISTO_ZL.Contig658.eannot.1268.final_new and Contig8.2.eannot.1185.final	*GH17*	Immunoreactive protein (unknown function)	1.0	10.2	1.1	1.0	18.4	3.3
HISTO_ZE.Contig158.eannot.1345.final_new and Contig14.7.eannot.1088.final	*CATB*	Catalase B, M antigen (stress response)	2.7	8.9	1.0	1.0	19.0	3.8
HISTO_ZZ.Contig127a.Fgenesh_Aspergillus.87.final_new and Contig9.8.eannot.1128.final		Superoxide dismutase (stress response)	2.8	19.4	1.0	1.0	7.5	2.4
HISTO_DM.Contig933.Fgenesh_histo.56.final_new and Contig3.2.Fgenesh_histo.7.final	*TSA1*	Thiol-specific antioxidant (stress response)	2.5	12.2	1.0	2.0	9.3	1.0
HISTO_ZL.Contig1131.Fgenesh_histo.235.final_new and Contig20.3.Fgenesh_histo.4.final		C2H2 finger domain-containing protein (transcription)	1.0	11.8	2.5	1.0	9.3	1.5
HISTO_EA.Contig33.eannot.1649.final_new and Contig12.20.eannot.1056.final		Nitroreductase (nitrogen metabolism)	1.2	11.9	1.0	1.0	8.4	2.5
HISTO_GY.Contig460.Fgenesh_Neurospora.78.final_new and Contig37.5.Fgenesh_Neurospora.3.final		Neutral amino acid permease (transport)	1.0	8.4	1.0	1.7	11.3	1.0
HISTO_BP.Contig457.Fgenesh_Aspergillus.167.final_new and Contig7.11.Fgenesh_Aspergillus.10.final		Histidine acid phosphatase (metabolism)	1.8	7.7	1.0	1.5	11.7	1.0
HISTO_JG.Contig207.eannot.1394.final_new and Contig6.25.eannot.1057.final	*GRX1*	Glutaredoxin (stress response)	1.0	8.7	1.7	2.1	9.3	1.0
HISTO_DY.Contig31.fgenesh_plus.50.final_new and Contig15.14.fgenesh_plus.25.final		Zinc-containing alcohol dehydrogenase (metabolism)	1.0	10.6	2.2	1.0	7.5	1.1
HISTO_JG.Contig206.eannot.1510.final_new and Contig24.6.eannot.1059.final	*SID3*	Acetylase (siderophore biosynthesis)	2.0	11.6	1.0	1.0	6.5	1.5

HISTO_FX.Contig167.Fgenesh_histo.44.final_new and Contig27.6.eannot.1031.final		Membrane transporter (transport)	2.9	12.7	1.0	1.0	5.5	1.1
HISTO_JG.Contig206.eannot.1556.final_new and Contig6.30.eannot.1215.final	*CAR1*	Arginase (metabolism)	1.0	9.7	2.9	1.0	6.5	1.0
HISTO_ZL.Contig1131.eannot.2134.final_new and Contig10.30.eannot.1086.final		Glucose transporter (transport)	1.0	9.6	1.0	1.0	5.4	1.0
HISTO_ZT.Contig181.Fgenesh_histo.190.final_new and Contig1.18.Fgenesh_Neurospora.4.final	*YMC1*	Mitochondrial carrier protein (metabolism)	1.0	6.8	1.0	1.0	7.5	2.5
HISTO_JG.Contig206.Fgenesh_histo.188.final_new and Contig24.6.Fgenesh_histo.10.final	*ABC1*	ABC multidrug transporter (iron transport)	1.1	9.4	1.0	1.2	4.7	1.0
HISTO_DF.Contig537.eannot.1356.final_new and Contig21.2-snap.4.final		Delta-1-pyrroline-5-carboxylate dehydrogenase PrnC (metabolism)	1.0	6.6	1.5	1.0	6.3	2.0
HISTO_ZL.Contig1158.Fgenesh_histo.83.final_new and Contig19.9.Fgenesh_histo.28.final		Peptidase family M13 protein, neprilysin, endothelin-converting enzyme (hydrolysis)	2.1	7.5	1.0	1.6	5.4	1.0
HISTO_ZL.Contig658.eannot.1234.final_new and Contig8.3.genewise.14.final	*CTS1*	Chitinase (cell wall)	2.0	9.3	1.0	1.1	3.9	1.0
HISTO_JG.Contig206.eannot.1512.final_new and Contig24.6.Fgenesh_histo.11.final	*SID1*	Ornithine monooxygenase (siderophore biosynthesis)	1.5	7.0	1.0	1.0	5.2	1.0
HISTO_ZT.Contig174.eannot.1401.final_new and Contig1.54.fgenesh_plus.4.final	*ADE4*	Amidophosphoribosyltransferase (metabolism)	2.3	7.6	1.0	1.0	4.3	1.2
HISTO_LF.Contig359.Fgenesh_Aspergillus.67.final_new and Contig2.49.Fgenesh_Aspergillus.1.final		Arrestin (cell signaling)	1.2	5.7	1.0	1.1	4.7	1.0
HISTO_GL.Contig233.Fgenesh_Neurospora.36.final_new and Contig4.20.Fgenesh_Neurospora.20.final		Glycosyl hydrolase catalytic core domain-containing protein (metabolism)	1.0	3.7	1.2	1.1	6.1	1.0
HISTO_DM.Contig933.eannot.1650.final_new and Contig1.62.eannot.1148.final	*RYP4*	C6 transcription factor, FacB (transcription)	1.3	5.6	1.0	1.0	4.0	1.0
HISTO_ZH.Contig107.eannot.1172.final_new and Contig5.3-snap.20.final	*FBP1*	Fructose-1,6-bisphosphatase (metabolism)	1.0	4.7	1.3	1.0	4.4	1.4
HISTO_ZT.Contig1128f.eannot.1145.final_new and Contig6.21.eannot.1022.final		AMP-dependent synthetase and ligase (metabolism)	1.0	4.3	1.4	1.1	4.5	1.0
Genes enriched in mycelia								
HISTO_GL.Contig296-snap.27.final_new and Contig1.70.eannot.1021.final		BYS1 (*Blastomyces* yeast-phase-specific protein) domain-containing protein (unknown function)	7.3	1.0	33.4	3.3	1.0	27.1
HISTO_LG.Contig392.eannot.1788.final_new and Contig22.4.eannot.1192.final	*MHB1*	Hydrophobin (cell wall)	7.6	1.0	35.2	4.6	1.0	16.0
HISTO_DF.Contig537.Fgenesh_histo.23.final_new and Contig21.2.Fgenesh_histo.29.final		Gluconolactonase (metabolism)	7.7	1.0	30.8	4.1	1.0	12.6
HISTO_EA.Contig33-snap.162.final_new and Contig12.20-snap.16.final	*VELC*	Velvet family protein (asexual development)	1.5	1.0	21.0	1.2	1.0	17.5
HISTO_ZL.Contig1131.Fgenesh_histo.234.final_new and Contig20.3.genewise.6.final		Extracellular serine-rich protein (cell wall)	2.7	1.0	19.8	1.7	1.0	17.5
HISTO_DM.Contig940.Fgenesh_histo.26.final_new and Contig4.1.eannot.1119.final		MFS monosaccharide transporter (transport)	2.6	1.0	21.0	2.9	1.0	13.5
HISTO_AZ.Contig47-snap.146.final_new and Contig2.22.eannot.1061.final	*ALD2*	Aldehyde reductase II (metabolism)	4.8	1.0	24.6	1.1	1.0	11.5

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TABLE 1 (Continued)

Systematic names (G217B and G186AR)[a]	Gene name	Annotation	Fold enrichment relative to lowest expression state[b]					
			G217B			G186AR		
			C	Y	M	C	Y	M
HISTO_ZL.Contig1161c.eannot.1522.final_new and Contig0.21.eannot.1019.final	*TYR1*	Tyrosinase	4.2	1.0	16.1	3.5	1.0	14.7
HISTO_AL.Contig317.Fgenesh_histo.177.final_new and Contig8.10.Fgenesh_histo.6.final	*PLD1*	Phospholipase D active site domain-containing protein	2.7	1.0	11.6	5.6	1.0	19.1
HISTO_DM.Contig93.Fgenesh_histo.142.final_new and Contig3.43.Fgenesh_histo.24.final		Binary toxin B domain-containing protein (unknown function)	1.4	1.0	16.0	1.2	1.0	13.8
HISTO_HS.Contig68.Fgenesh_histo.222.final_new and Contig3.88.Fgenesh_histo.17.final	*EFG1/STU1*	APSES basic helix-loop-helix transcription factor (transcription)	3.2	1.0	15.6	2.2	1.0	14.1
HISTO_ZE.Contig158.eannot.1424.final_new and Contig14.7.Fgenesh_Neurospora.18.final		FAD binding domain-containing protein (metabolism—unknown function)	3.8	1.0	12.8	1.1	1.0	16.5
HISTO_KF.Contig470.Fgenesh_histo.2.final_new and Contig11.28.Fgenesh_Aspergillus.26.final	*CTS3*	Chitinase (cell wall metabolism)	2.9	1.0	13.2	3.7	1.0	15.4
HISTO_DM.Contig933.Fgenesh_histo.24.final_new and Contig3.5.Fgenesh_histo.2.final		1,4-Alpha-glucan branching enzyme domain, GlgB (cell wall modification)	3.2	1.0	11.8	2.8	1.0	16.3
HISTO_ZE.Contig158-snap.6.final_new and Contig14.15.Fgenesh_Aspergillus.3.final		C2H2 finger domain-containing protein (transcription)	2.2	1.0	14.0	2.1	1.0	12.1
HISTO_DY.Contig31-snap.10.final_new and Contig15.15-snap.13.final		ThiJ/PfpI family protein	2.3	1.0	17.9	2.2	1.0	9.3
HISTO_ZT.Contig1129.eannot.1103.final_new and Contig1.33.eannot.1103.final		Protein tyrosine/serine phosphatase domain protein (cell signaling)	2.6	1.0	14.5	1.3	1.0	10.5
HISTO_DM.Contig933.eannot.1555.final_new and Contig1.59.eannot.1036.final	*ELI1*	ELI-ag1-like protein, expression library immunization antigen 1 (host interaction)	1.0	8.0	24.8	1.5	1.0	6.1
HISTO_FE.Contig19-snap.201.final_new and Contig0.43-snap.6.final		Glycosylphosphatidylinositol (GPI)-anchored serine-threonine rich protein (cell wall)	1.0	3.5	12.5	1.0	2.6	11.8
HISTO_GI.Contig382.Fgenesh_histo.82.final_new and Contig2.67.Fgenesh_histo.9.final		Fasciclin domain-containing protein (unknown function)	1.5	1.0	13.4	1.2	1.0	10.6
HISTO_GL.Contig296.Fgenesh_Aspergillus.79.final_new and Contig1.69.Fgenesh_histo.14.final		Salicylate hydroxylase, FAD binding domain-containing protein	1.1	1.0	14.4	1.0	1.4	9.5
HISTO_ZZ.Contig127c.eannot.1552.final_new and Contig13.21.eannot.1014.final		SCP-like extracellular protein (unknown function)	1.9	1.0	13.3	1.0	1.4	9.2
HISTO_ZL.Contig1161c.eannot.1487.final_new and Contig0.12.Fgenesh_histo.21.final		Ctr copper transporter (transport)	2.3	1.0	12.1	2.5	1.0	10.1
HISTO_LG.Contig392.eannot.1695.final_new and Contig22.3.Fgenesh_Aspergillus.4.final		MFS transporter (transport)	1.8	1.0	10.3	1.8	1.0	11.0
HISTO_JG.Contig207.Fgenesh_histo.89.final_new and Contig6.30.Fgenesh_histo.55.final		Chromosome segregation ATPase family protein	1.3	1.0	11.6	1.2	1.0	9.4
HISTO_HS.Contig68.Fgenesh_histo.200.final_new and Contig3.88.Fgenesh_histo.6.final		C6 zinc finger domain-containing protein (transcription)	1.0	1.4	11.8	1.0	1.1	8.4
HISTO_BP.Contig457.Fgenesh_histo.177.final_new and Contig7.4.Fgenesh_Aspergillus.2.final		Proline-specific permease (amino acid transport)	1.3	1.0	5.0	1.0	1.1	18.7
HISTO_ZT.Contig174.eannot.1389.final_new and Contig6.15.eannot.1092.final		Tc5 transposase DNA-binding domain, CENPB domain (Tn transcription)	1.0	1.1	6.5	1.0	1.1	13.3

HISTO_BP.Contig457.Fgenesh_Aspergillus.216.final_new and Contig7.4.Fgenesh_Aspergillus.58.final	*CCC2*	Calcium transporter	2.1	1.0	8.0	1.9	1.0	9.9
HISTO_ZE.Contig158.Fgenesh_Aspergillus.25.final_new and Contig14.7-snap.12.final		SAM-dependent methyltransferase (metabolism)	1.3	1.0	9.6	1.2	1.0	7.8
HISTO_ZL.Contig1161a.eannot.1162.final_new and Contig0.35.eannot.1060.final		MFS monocarboxylate transporter (transport)	1.8	1.0	12.1	1.1	1.0	6.1
HISTO_ZE.Contig158.eannot.1426.final_new and Contig14.7.eannot.1095.final		MFS multidrug transporter (transport—drug resistance)	2.1	1.0	7.7	3.1	1.0	9.6
HISTO_BP.Contig459.Fgenesh_histo.160.final_new and Contig30.15.fgenesh_plus.20.final		C2H2 finger domain-containing protein (transcription)	1.0	1.3	5.2	1.7	1.0	14.0
HISTO_ZL.Contig1161c.Fgenesh_histo.47.final_new and Contig0.19.Fgenesh_histo.2.final		Morphogenesis-related protein, Rho-GAP superfamily domain (cell signaling)	1.4	1.0	8.3	1.0	1.2	8.5
HISTO_BP.Contig457.Fgenesh_Aspergillus.17.final_new and Contig7.18.genewise.32.final		RNA-binding protein Nrd1, negative regulator of differentiation (asexual development)	1.0	1.6	7.8	1.0	1.8	9.1
HISTO_ZL.Contig658.Fgenesh_Aspergillus.66.final_new and Contig8.3.Fgenesh_Aspergillus.24.final	*NIT88*	PT repeat family protein, RNase E domains	1.0	1.2	7.0	2.8	1.0	9.7
HISTO_FE.Contig19.eannot.1909.final_new and Contig0.49.eannot.1124.final	*FBC1*	C2H2 finger domain-containing protein FlbC (transcription)	1.0	1.1	6.3	1.4	1.0	10.4
HISTO_ZT.Contig174.Fgenesh_histo.17.final_new and Contig1.50.Fgenesh_histo.1.final	*PUM2*	mRNA-binding protein Pumilio 2 (RNA metabolism)	1.0	2.0	10.6	1.6	1.0	5.7
REPEAT NAA.Contig17f.Fgenesh_histo.4.final_new and Contig11.4.Fgenesh_histo.7.final		Bifunctional P-450:NADPH-P450 reductase (metabolism)	1.8	1.0	5.6	1.4	1.0	10.4
HISTO_HS.Contig68-snap.7.final_new and Contig3.49.Fgenesh_histo.5.final		Protein kinase (protein phosphorylation—cell signaling)	1.0	1.2	5.1	2.8	1.0	11.5
HISTO_ZL.Contig1131-snap.217.final_new and Contig10.27-snap.8.final	*MSB2*	Mucin family signaling protein, putative (cell signaling)	1.0	1.0	7.5	1.0	1.0	7.6
HISTO_IAH.Contig38.eannot.1006.final_new and Contig20.3-snap.8.final	*FDH1*	Formate dehydrogenase (metabolism)	1.7	1.0	6.2	1.4	1.0	8.9
HISTO_BP.Contig457-snap.10.final_new and Contig7.4-snap.21.final	*BSP1*	PBSP domain-containing protein, plant basic secretory protein	1.2	1.0	5.6	1.0	1.0	8.8
HISTO_DY.Contig31.Fgenesh_Aspergillus.39.final_new and Contig15.6.eannot.1059.final		Secretory phospholipase A2 (lipid metabolism)	1.0	1.3	4.9	2.1	1.0	10.0
HISTO_ZU.Contig153f.Fgenesh_histo.5.final_new and Contig17.12-snap.1.final		AT DNA-binding protein (transcription)	1.1	1.0	6.3	2.6	1.0	7.7
HISTO_DM.Contig933.Fgenesh_histo.74.final_new and Contig3.2.Fgenesh_histo.1.final		Basic region leucine zipper, bZIP transcription factor (transcription)	1.0	1.0	8.6	1.0	1.1	4.8
HISTO_KK.Contig134.Fgenesh_Aspergillus.17.final_new and Contig1.3-snap.11.final		Phosphoribulokinase/uridine kinase family protein (metabolism)	1.0	1.2	4.3	1.9	1.0	8.7
HISTO_ZL.Contig1161d-snap.23.final_new and Contig0.5-snap.37.final		Lactone hydrolase (metabolism—hydrolysis)	1.1	1.0	3.6	1.3	1.0	10.2
HISTO_DA.Contig93.Fgenesh_histo.176.final_new and Contig0.98.Fgenesh_histo.19.final		Calcium/calmodulin-dependent protein kinase	1.3	1.0	5.6	1.7	1.0	6.3
HISTO_ZZ.Contig127a.Fgenesh_histo.112.final_new and Contig9.8.Fgenesh_Neurospora.18.final		Asparagine synthase (metabolism)	1.3	1.0	4.0	2.0	1.0	8.5
HISTO_ZL.Contig1161b.eannot.1356.final_new and Contig0.29.eannot.1064.final	*ESDC*	GTP-binding protein (sexual development)	1.2	1.0	7.6	1.0	1.0	4.4

(Continued on following page)

TABLE 1 (Continued)

Systematic names (G217B and G186AR)[a]	Gene name	Annotation	Fold enrichment relative to lowest expression state[b]					
			G217B			G186AR		
			C	Y	M	C	Y	M
HISTO_GL.Contig233.fgenesh_plus.16.final_new and Contig4.24.fgenesh_plus.2.final	*IME2*	Sporulation protein kinase (sexual development, protein phosphorylation, cell signaling)	1.0	1.0	7.2	1.0	1.4	4.2
HISTO_DM.Contig933-snap.26.final_new and Contig1.62-snap.6.final		Cell wall proline-rich protein (cell wall)	1.0	1.2	6.1	1.0	1.1	4.6
HISTO_HS.Contig68.eannot.2023.final_new and Contig3.65.eannot.1186.final	*FLU1*	Developmental protein FluG (asexual development)	1.0	1.1	4.6	1.0	1.0	4.6
HISTO_ZT.Contig1089.eannot.1615.final_new and Contig1.43.eannot.1299.final		PLAC8 family protein (unknown function)	1.3	1.0	5.1	1.0	1.0	4.1
HISTO_AL.Contig317.eannot.1462.final_new and Contig8.16.eannot.1029.final		Uracil phosphoribosyltransferase (metabolism)	1.6	1.0	5.1	1.0	1.0	3.3
HISTO_HS.Contig68.fgenesh_plus.53.final_new and Contig3.64.Fgenesh_histo.8.final		Septin, cell division protein Sep4a (cell wall—cell division)	1.0	1.0	4.3	1.2	1.0	3.7
HISTO_DU.Contig190.Fgenesh_Neurospora.39.final_new and Contig13.13-snap.2.final		Myosin class II heavy chain (cell structure)	1.0	1.1	3.3	1.0	1.2	4.3

[a] For genes enriched in conidia, there were 27 genes for conserved proteins of unknown function and 6 genes for proteins of unknown function, as well as 9 genes with no BLAST hits. For genes enriched in yeast, there were 4 genes for conserved proteins of unknown function and 6 genes for proteins of unknown function. For genes enriched in mycelia, there were 24 genes for conserved proteins of unknown function and 3 genes for proteins of unknown function, as well as 1 gene with no BLAST hits.

[b] C, conidia; Y, yeast cells; M, mycelia.

conidial enriched genes in G217B and G186AR, respectively; 86 of these genes showed conserved differential expression in the two strains. There were 385 and 350 mycelial enriched genes in G217B and G186AR, respectively, with 86 of these genes showing conserved expression. There were 175 and 159 yeast enriched genes in G217B and G186AR, respectively, with 45 of these genes showing conserved expression. Numerous strain-specific differential genes did not fall into the conserved expression set because they did not quite meet the criterion of 3-fold enriched expression in one phase over both of the remaining phases (selected genes are shown in Table 2). To validate phase-specific enriched expression, a representative gene was chosen for each category (Fig. 5). We used Northern blotting or reporter gene technology to determine that *CATA*, *TYR1*, and *CBP1* (all described below) did indeed show phase-specific enriched expression.

Conidial enriched transcripts are predicted to be involved in stress responses, DNA metabolism, transcriptional regulation, and pathogenesis. Conidia expressed a large number of predicted genes with unknown functions or without BLASTP matches in the NCBI nr database. Conidial enriched genes were also implicated in resistance to stress, trehalose metabolism, DNA repair, signal transduction, asexual development, and host interactions. Catalase A is a conidium-specific catalase in *A. nidulans* and *A. fumigatus* that is induced by stress conditions and confers increased resistance to hydrogen peroxide stress (26, 27). We consistently observed very high levels of expression of catalase A (*CATA*) in *H. capsulatum* conidia. To validate this expression pattern of *CATA* in *H. capsulatum*, we performed Northern blot analysis on conidial, mycelial, and yeast total RNAs and found that *CATA* was indeed expressed most highly in conidia (Fig. 5). Interestingly, our microarray experiments revealed that the unnamed gene adjacent to *CATA* (Contig1.53.Fgenesh_histo.12.final in the G186AR strain and HISTO_ZT.Contig174.Fgenesh_histo.8.final_new in the G217B strain) also showed enriched expression in conidia and is transcribed in the opposite direction from *CATA*. These findings suggest that *CATA* and this gene of unknown function share a bidirectional, conidia-specific promoter that is conserved between both strains of *H. capsulatum*.

Other *H. capsulatum* conidial enriched transcripts are predicted to encode orthologs of stress-responsive factors from other fungi. Examples include the ortholog of *rds1*, which is induced in *Schizosaccharomyces pombe* by temperature shift, specific nutrient limitation, or other stresses (28). Additionally, *H. capsulatum* conidia displayed higher levels of a transcript that is predicted to encode the ortholog of Ish1, which is essential for stationary-phase viability of *S. pombe* (29). Similarly, trehalose is a common storage and stress resistance carbohydrate in fungal spores, and *H. capsulatum* spores showed upregulation of transcripts implicated in trehalose metabolism. The transcript encoding alpha, alpha-trehalose phosphate synthase subunit 1, *TPS1*, was enriched in G217B conidia (Table 2), whereas *TPS3* was enriched in conidia of both strains (Table 1). The transcript encoding a putative glutamate decarboxylase, *GAD1* (Table 1), was also highly enriched in conidia. In *S. cerevisiae*, *GAD1* is required for tolerance to oxidative stress (30), and in *Neurospora crassa*, it is a developmentally regulated enzyme that is responsible for catalyzing the first step in the metabolism of the large pool of free glutamic acid during conidial germination (31) (Table 1).

Conidial enriched transcripts included a number of genes involved in DNA repair, such as the transcript predicted to encode

the nonhomologous end-joining (NHEJ) DNA ligase Dnl4. Transcripts encoding an ortholog of Spo11 (a meiosis-specific enzyme that catalyzes the formation of double-strand breaks in DNA) and an ortholog of Pri1 (a DNA primase small subunit involved in DNA replication) were enriched in G217B conidia, although the role of the Spo11 ortholog in vegetative conidia is unclear. Overall, these results suggest that conidia of both strains are equipped for DNA repair and modification, a property that may make these cells more resistant to environmental insults such as UV exposure. Notably, the differential ability of conidia, yeast, and mycelia to cope with stress has not been assessed in laboratory strains of *H. capsulatum*.

In many fungi, conidiation is under light control; for example, both *N. crassa* and *A. nidulans* conidiate in response to light signals. *H. capsulatum* conidia expressed transcripts predicted to encode orthologs of three putative light-responsive receptor proteins: Ops1, an opsin; and Whc1 and Whc2, orthologs of the white collar-1 and white collar-2 proteins, respectively. The *N. crassa* opsin gene, *nop-1*, encodes a putative green-light photoreceptor that is highly expressed in cultures that support conidiation (32). *wc-1* and *wc-2* form a blue-light sensing White Collar complex that regulates the expression of hundreds of *N. crassa* genes (33, 34), including at least 24 downstream transcription factors (35). Little is known about the role of light in conidiation of *H. capsulatum*, although it does conidiate under either light or dark conditions. The upregulation of these transcripts in *H. capsulatum* suggests that a functional White Collar complex exists in conidia and may regulate conidial gene expression. Interestingly, in *A. nidulans*, both FlbD and FluG are induced in response to light (36). In *H. capsulatum*, a transcript encoding the ortholog of FlbD (*FBD1*) was also preferentially expressed in conidia (Table 1), although *FLU1* (the *H. capsulatum* counterpart of *A. nidulans* FluG) was preferentially expressed in mycelia (Table 1).

Since conidia are the natural infectious particle of *H. capsulatum*, we were particularly interested in factors with the potential to influence pathogenesis and/or host interactions. For example, the transcript predicted to encode the *H. capsulatum* ortholog of Cap20 was expressed predominantly in G217B conidia, whereas the G186AR ortholog was expressed in both conidia and yeast. Cap20 is a putative perilipin family protein whose message has been detected at the leading edge of the infection front in tomato fruits infected with the plant fungal pathogen *Colletotrichum gloeosporioides*. A *cap20* mutant of *C. gloeosporioides* has decreased virulence on avocado and tomato fruits, suggesting that Cap20 plays a role in host infection (37). Additionally, the transcript predicted to encode Pth11, an integral membrane G-protein-coupled receptor (38), was preferentially expressed in conidia of the G217B strain. Pth11 from the plant fungal pathogen *Magnaporthe grisea* is a novel plasma membrane protein that mediates differentiation of the *M. grisea* appressorium structure required for virulence (39).

Finally, G217B and G186AR conidial enriched transcripts included some encoding both shared and unique sets of transcription factors, most with unknown functions (Tables 1 and 2). These transcription factors included predicted C2H2 domain-containing proteins, Zn(II)2Cys6 zinc binuclear cluster domain transcription factors, and NmrA- and NmdA-like transcription factors. These uncharacterized transcription factors might regulate conidium-specific functions, including germination. Additionally, we made the surprising observation that the Ryp1 transcriptional regulator, which was previously observed to be expressed differentially in *H. capsulatum* yeast compared to mycelia and is absolutely required for yeast-phase growth (13), is almost as highly expressed in G217B conidia as it is in yeast cells (2.67-fold higher in conidia than in mycelia and 2.74-fold higher in yeast than in mycelia). This unexpected expression pattern suggests a role for Ryp1 in conidial biology, in addition to its role in yeast-phase growth. Indeed, conidial viability defects were observed in *ryp1* mutants in culture (V. Q. Nguyen and D. O. Inglis, data not shown).

Mycelial enriched transcripts include those implicated in gene expression, development, mating, and translation. We identified 385 transcripts that were differentially expressed in G217B mycelia compared to conidia and yeast cells and 350 transcripts that were preferentially enriched in the mycelia of G186AR. Mycelial enriched transcripts included those predicted to encode regulatory factors. Notably, orthologs of these proteins in *A. nidulans* included transcription factors involved in orchestrating a conserved asexual development pathway (FlbC, WetA, ApsA, and StuA) (40) or the balance between sexual and asexual spore formation (EsdC and VelC) (40, 41). Factors predicted to regulate morphology, such as orthologs of Msb2 (42) and a morphogenesis-related Rho-GAP (43), were also preferentially expressed in mycelia, as were a significant number of predicted genes with unknown functions.

fluG and *flbC* are known as *fluffy* genes in *A. nidulans*, because of the colony morphology of the corresponding mutants. Whereas wild-type hyphae of *A. nidulans* form elaborate developmental structures (conidiophores) in which vegetative spores develop, these *A. nidulans fluffy* mutants form excessive aerial hyphae that lack conidiophores (44). These genes are part of a well-characterized, conserved regulatory pathway that governs light-induced conidiophore development and conidium production in *Aspergillus*. The *H. capsulatum FLU1* transcript, which is predicted to encode the ortholog of FluG, and the *H. capsulatum FBC1* transcript, which is predicted to encode the ortholog of FlbC, were enriched in mycelia in G217B and G186AR. Additionally, we observed enriched expression of the transcripts predicted to encode the orthologs of *A. nidulans* WetA (*H. capsulatum WET1*) and *A. nidulans* ApsA (*H. capsulatum APS1*). *wetA* is a late-stage-induced conidiation gene implicated in *A. nidulans* spore wall biogenesis (45–47), and *A. nidulans apsA* is required for the proper positioning of nuclei within conidiophores and hyphae (48). The *H. capsulatum* ortholog of BrlA, the master regulator that initiates conidiophore formation in *A. nidulans*, was not detected in our microarrays.

Members of the Asm1, Phd1, Sok2, Efg1, and StuA (APSES) family of fungal proteins regulate morphogenesis and virulence in ascomycetes (49). We observed enriched expression in mycelia of a transcript predicted to encode the *H. capsulatum* APSES ortholog Efg1. The *A. fumigatus* APSES ortholog, StuA, is markedly upregulated after hyphal cells acquire the ability to form conidia (developmental competence) and is required for appropriate conidiation and conidiophore morphology (49). In *C. albicans*, the APSES ortholog Efg1 is required for filamentous growth (50, 51). *H. capsulatum EFG1* showed enriched expression in mycelia of both strains (Table 1).

Finally, we observed enriched expression in mycelia for the transcript encoding the ortholog of *A. nidulans* VelC. The VelC protein is a member of a protein family of four *A. nidulans* factors, VeA, VelB, VelC, and VosA, that share a "velvet" do-

TABLE 2 Selected strain-specific differentially expressed transcripts of strains G186AR and G217B

Systematic name	Gene name	Annotation	Ortholog detected[a]	Fold enrichment relative to lowest expression state[b]					
				G217B			G186AR		
				C	Y	M	C	Y	M
Selected G217B genes enriched in conidia									
HISTO_DA.Contig93.Fgenesh_histo.280.final_new	*TYR4*	Tyrosinase (melanin biosynthesis)	Y	21.5	1.0	6.2	14.0	1.0	9.5
HISTO_GL.Contig233-snap.6.final_new	*LSP1*	Sphingolipid long-chain base-responsive inhibitor of protein kinase PIL1/LSP1	Y	20.7	1.1	1.0	14.2	5.5	1.0
HISTO_ZU.Contig65.fgenesh_plus.16.final_new	*NCE102*	Nonclassical export protein	Y	20.0	2.3	1.0	1.3	1.3	1.0
HISTO_ZU.Contig65.Fgenesh_histo.143.final_new		Glyoxalase (metabolism)	Y	14.6	1.0	1.3	5.8	1.0	3.3
HISTO_ZE.Contig158.eannot.1405.final_new		NmrA family transcriptional regulator (transcription)	Y	14.2	2.0	1.0	5.9	2.2	1.0
HISTO_JG.Contig206.eannot.1472.final_new	*PDC1*	Pyruvate decarboxylase	Y	13.7	3.4	1.0	7.7	3.7	1.0
HISTO_DM.Contig93-snap.23.final_new	*WHC2*	Zinc finger white collar 2 light sensor (transcription)	Y	12.7	1.8	1.0	2.6	1.0	1.5
HISTO_AZ.Contig47.eannot.1654.final_new	*NDH1*	Pyridine nucleotide-disulfide oxidoreductase	N	11.0	1.0	2.6			
HISTO_AZ.Contig47-snap.112.final_new	*PTH11*	Pth11-like integral membrane protein (pathogenesis)	Y	10.7	1.0	2.5	10.6	1.0	4.6
HISTO_ZZ.Contig2f.Fgenesh_histo.14.final_new		C6 transcription factor (transcription)	Y	10.0	1.0	1.2	5.0	2.8	1.0
HISTO_DM.Contig933-snap.69.final_new	*NIT25*	CobW domain-containing protein	Y	9.9	2.2	1.0	10.3	6.6	1.0
HISTO_KF.Contig597.Fgenesh_Aspergillus.127.final_new	*LDF2*	Lysis defective 2, no significant blast hit (unknown function)	N	9.5	1.5	1.0			
HISTO_AL.Contig317.Fgenesh_histo.57.final_new	*GAP1*	General amino acid permease (transport)	Y	9.1	1.5	1.0	8.3	4.0	1.0
HISTO_GY.Contig460.eannot.1661.final_new		Gal4-like DNA-binding domain-containing protein (transcription)	N	8.7	1.8	1.0			
HISTO_LG.Contig392.eannot.1727.final_new		Superoxide dismutase (stress response)	N	8.6	1.0	2.8			
HISTO_BP.Contig459.eannot.1565.final_new	*AQY1*	Aquaporin	Y	8.4	2.0	1.0	5.5	5.4	1.0
HISTO_DF.Contig537.Fgenesh_histo.90.final_new		C6 transcription factor (transcription)	Y	8.2	1.0	1.3	1.7	1.0	1.7
HISTO_BP.Contig459.Fgenesh_histo.49.final_new	*PSD2*	Phosphatidylserine decarboxylase proenzyme	Y	7.7	1.9	1.0	3.3	1.0	2.0
HISTO_EA.Contig33.Fgenesh_histo.236.final_new		Regulatory protein, C2H2 transcription factor (AmdA) (transcription)	Y	7.6	1.0	1.6	9.6	1.0	4.0
HISTO_ZU.Contig65.genewise.88.final_new		DNA primase small subunit (DNA metabolism—DNA repair)	Y	7.6	1.9	1.0	1.0	2.5	2.6
HISTO_ZZ.Contig2f.Fgenesh_histo.16.final_new	*NIT79*	Nitrosative stress-induced transcript (stress response)	Y	7.5	1.0	1.1	3.5	1.4	1.0
HISTO_ZT.Contig1129.eannot.1089.final_new	*SUR7*	Actin cortical patch protein, putative (stress response)	Y	6.8	1.5	1.0	2.0	1.0	1.1
HISTO_ZZ.Contig127c-snap.30.final_new	*NRK1*	Nicotinamide riboside kinase	Y	6.2	2.0	1.0	1.0	1.8	1.7
HISTO_LG.Contig392-snap.118.final_new	*SPO11*	Sexual or parasexual topoisomerase (DNA metabolism)	Y	5.9	1.0	1.8	1.0	1.4	1.1
HISTO_GY.Contig460.eannot.1775.final_new		C2H2-type zinc finger transcription factor (transcription)	Y	5.8	1.0	1.6	3.0	1.4	1.0
HISTO_GI.Contig382.eannot.1253.final_new	*NIT69*	Nitrosative stress-induced transcript (stress response)	N	5.6	1.0	1.5			

HISTO_ZT.Contig1089.fgenesh_plus.107.final_new	*TPS1*	Alpha,alpha-trehalose-phosphate synthase 1 (trehalose metabolism)	Y	5.5	1.7	1.0	2.1	1.4	1.0
HISTO_LF.Contig359.Fgenesh_Aspergillus.171.final_new		HMG box domain-containing protein (transcription)	Y	5.3	1.0	1.5			
HISTO_ER.Contig17.Fgenesh_histo.79.final_new		C6 transcription factor (transcription)	Y	5.1	1.0	1.2	3.3	1.0	1.7
HISTO_GL.Contig296-snap.22.final_new	*RAP1*	Transcription factor (transcription)	Y	4.9	1.0	1.2	2.0	1.6	1.0
HISTO_KF.Contig597.Fgenesh_histo.87.final_new	*NIT76*	RING finger protein	Y	4.9	1.0	1.3	3.6	2.6	1.0
HISTO_ZL.Contig658.Fgenesh_Aspergillus.62.final_new		QDE-2-interacting protein (RNA interference)	Y	4.7	1.0	1.4	3.9	1.0	1.5
HISTO_ZL.Contig658.eannot.1282.final_new	*CAP20*	Perilipin family pathogenesis-associated protein (pathogenesis)	Y	4.4	1.3	1.0	2.8	2.4	1.0
HISTO_ZE.Contig158.eannot.1339.final_new		Antibiotic synthetase, NRPS-like enzyme (secondary metabolism)	Y	4.4	1.0	1.3	1.0	3.9	3.0
HISTO_EA.Contig33.genewise.135.final_new	*ECM15*	Cell wall biogenesis protein Ecm15	Y	4.3	1.4	1.0	2.6	1.9	1.0
HISTO_DA.Contig93.Fgenesh_Neurospora.21.final_new	*GAC1*	Protein phosphatase regulatory subunit (protein dephosphorylation)	Y	3.9	1.0	1.0	1.0	4.2	1.1
HISTO_LF.Contig359.Fgenesh_histo.207.final_new		C6 transcription factor (transcription)	Y	3.9	1.0	1.2	3.2	1.0	2.1
HISTO_ZY.Contig518.Fgenesh_histo.47.final_new		Mitogen-activated protein (MAP) kinase Hog1 (cell signaling)	Y	3.9	1.1	1.0	1.7	1.5	1.0
HISTO_AZ.Contig47.Fgenesh_histo.82.final_new		C6 transcription factor (transcription)	Y	3.5	1.0	1.1	1.4	1.0	1.9
HISTO_ZT.Contig181-snap.98.final_new		Glyoxalase family protein (metabolism)	Y	3.5	1.0	1.1			
Selected G217B genes enriched in yeast									
HISTO_GL.Contig296.eannot.1366.final_new	*LDF1*	Lysis defective 1 (unknown function)	Y	1.0	96.4	1.2			
HISTO_ZZ.Contig127a.eannot.1408.final_new		Zinc responsiveness transcriptional activator (transcription)	Y	2.5	29.1	1.0	1.0	1.9	1.0
HISTO_AZ.Contig47.Fgenesh_histo.160.final_new	*MFS1*	Siderophore-iron transporter (iron transport)	Y	1.0	26.8	8.2	1.0	3.1	5.3
HISTO_ZL.Contig1117.eannot.1110.final_new	*CBP1*	Calcium-binding protein precursor (unknown function—pathogenesis)	Y	1.3	22.7	1.0	1.0	2.2	1.3
HISTO_AL.Contig317.eannot.1560.final_new	*YPS3*	Yeast-phase-specific protein (unknown function)	Y	1.9	22.1	1.0	7.2	1.0	21.8
HISTO_ZL.Contig1161b.fgenesh_plus.67.final_new	*CYP51*	Cytochrome P450 sterol 14 alpha-demethylase	Y	1.0	20.5	6.3	13.9	1.0	14.7
HISTO_ZT.Contig174.Fgenesh_histo.111.final_new	*CDO1*	Cysteine dioxygenase (cysteine catabolism)	Y	1.0	19.1	3.5	1.0	7.9	2.9
HISTO_FX.Contig167.eannot.1297.final_new		Lysis defective 1 (LDF1) paralog (unknown function)	N	2.4	16.8	1.0			
HISTO_ZL.Contig1131.Fgenesh_histo.321.final_new	*USP107*	U1 snRNP-associated protein Usp107	Y	1.2	12.7	1.0	1.0	2.1	1.0
HISTO_ZL.Contig1161c.fgenesh_plus.4.final_new		Nitrogen regulatory protein OTam, C6 transcription factor (transcription)	Y	1.7	11.5	1.0			
HISTO_GY.Contig460.eannot.1805.final_new	*CPH1*	Transcription factor SteA (sexual development)	Y	1.0	11.1	2.8	1.0	11.4	4.4

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TABLE 2 (Continued)

Systematic name	Gene name	Annotation	Ortholog detected[a]	Fold enrichment relative to lowest expression state[b]					
				G217B			G186AR		
				C	Y	M	C	Y	M
HISTO_ZL.Contig1131.eannot.1954.final_new	*CATP*	Catalase isozyme P (stress resistance)	Y	1.0	8.0	1.2	1.0	2.6	2.0
HISTO_DF.Contig537.Fgenesh_histo.19.final_new	*GEL2*	Beta-glucanosyltransferase	Y	2.1	8.0	1.0	2.3	6.2	1.0
HISTO_ZL.Contig1131.Fgenesh_histo.184.final_new		C6 finger domain-containing protein (transcription)	Y	2.4	7.5	1.0	2.9	1.0	1.4
HISTO_FE.Contig19.eannot.1724.final_new	*ARO8*	Aromatic amino acid aminotransferase Aro8	Y	1.0	7.1	1.0	1.0	1.3	2.8
HISTO_GB.Contig114.fgenesh_plus.17.final_new	*CHK2*	Serine/threonine protein kinase (protein phosphorylation—cell signaling)	Y	1.0	7.1	1.9	1.0	1.8	1.5
HISTO_LG.Contig392.Fgenesh_histo.47.final_new	*DIP5*	Amino acid permease (transport)	Y	1.4	7.1	1.0	2.5	1.6	1.0
HISTO_DM.Contig93.eannot.1715.final_new	*NIT55*	Conserved protein of unknown function, nitrosative stress-induced transcript	Y	1.0	6.4	1.3	1.0	3.8	1.5
HISTO_GI.Contig382.eannot.1300.final_new	*ACH1*	Acyl-CoA carboxylate CoA-transferase Ach1	Y	1.2	5.5	1.0			
HISTO_ZU.Contig65.Fgenesh_histo.202.final_new	*POS5*	Poly(p)/ATP NAD kinase POS5	Y	1.2	5.4	1.0	1.0	2.2	1.5
HISTO_ZY.Contig571f.Fgenesh_histo.21.final_new		C2HC5 zinc finger domain-containing protein (protein interaction)	Y	1.1	5.1	1.0	1.0	1.4	1.3
HISTO_GI.Contig363.eannot.1195.final_new	*STT3*	Oligosaccharyltransferase Stt3 subunit	Y	1.0	4.6	1.5	1.0	4.0	2.5
HISTO_ZH.Contig107.eannot.1171.final_new	*OSM1*	Fumarate reductase flavoprotein subunit	Y	1.0	4.0	1.3	1.6	1.6	1.0
HISTO_DA.Contig93.Fgenesh_histo.81.final_new		GAL4 and fungus-specific transcription factor domain-containing protein PriB (transcription)	Y	1.1	3.9	1.0	1.6	2.6	1.0
HISTO_BP.Contig459.Fgenesh_histo.154.final_new	*ANP1*	Mannosyltransferase complex subunit Anp1, aminonitrophenyl propanediol resistance protein	Y	1.0	3.9	1.3	1.0	3.1	1.6
HISTO_ZL.Contig1161b.Fgenesh_Aspergillus.104.final_new		Histone deacetylase HosA (transcription)	Y	1.0	3.8	1.0	1.0	3.1	1.8
HISTO_ZZ.Contig127c.Fgenesh_histo.29.final_new	*RYP2*	Velvet family protein, ortholog of *A. nidulans* VosA (morphogenesis)	Y	1.0	3.8	1.1	1.7	4.1	1.0
HISTO_ZT.Contig174.Fgenesh_Aspergillus.43.final_new	*RBG1*	Developmentally regulated GTP-binding protein (asexual development)	Y	1.0	3.7	1.2	1.0	2.2	2.5
HISTO_ZU.Contig65.Fgenesh_histo.97.final_new	*GUF1*	GTP-binding protein, translation factor GUF1, mitochondrial	Y	1.1	3.5	1.0	1.4	1.5	1.0
HISTO_HS.Contig68.Fgenesh_histo.264.final_new	*UTR1*	NAD kinase associated with ferric reductase Utr1	Y	1.1	3.4	1.0	1.0	2.7	1.6
HISTO_GL.Contig59.eannot.1284.final_new	*FNX1*	Multidrug resistance protein Fnx1	Y	1.0	3.1	1.0	1.2	1.0	4.1
Selected G217B genes enriched in mycelia									
HISTO_LL.Contig1.Fgenesh_histo.8.final_new	*DOC1*	DOC family protein	N	7.7	1.0	32.9			
HISTO_GY.Contig460.eannot.1710.final_new	*RPS23*	Ribosomal protein S23	Y	1.0	1.7	23.3	1.0	2.7	3.1
HISTO_ZZ.Contig127c.eannot.1499.final_new		HMG box transcription factor, similar to mating type protein MAT1-2, not part of MTL (transcription)	Y	3.2	1.0	22.9	4.9	1.0	1.6

HISTO_ZZ.Contig127c.Fgenesh_histo.55.final_new		HMG box transcription factor, similar to mating type protein MAT1-2, not part of MTL (transcription)	Y	1.8	1.0	21.0	5.4	1.0	8.7
HISTO_EA.Contig33.eannot.1633.final_new	*MAT1-1*	Alpha-box mating type protein of the mating type locus (transcription)	N	2.7	1.0	14.4			
HISTO_AL.Contig317-snap.27.final_new	*TYR6*	Tyrosinase (melanin biosynthesis)	Y	2.1	1.0	12.4			
HISTO_DM.Contig93-snap.72.final_new	*WET1*	Developmental regulatory protein WetA (cell wall)	Y	1.7	1.0	11.0			
HISTO_LF.Contig359.Fgenesh_histo.200.final_new	*NIT66*	Conserved protein of unknown function	Y	1.4	1.0	10.8	1.5	1.0	1.1
HISTO_GY.Contig471.Fgenesh_Aspergillus.61.final_new		C2H2 finger domain-containing protein (transcription)	N	3.4	1.0	10.7			
HISTO_ZL.Contig1131.Fgenesh_Aspergillus.295.final_new		NDT80/PhoG-like DNA-binding family protein (transcription)	Y	2.3	1.0	10.2	3.5	1.0	1.1
HISTO_LF.Contig359.Fgenesh_histo.82.final_new		C2H2 zinc finger domain-containing protein (transcription)	Y	1.2	1.0	9.9			
HISTO_GP.Contig38.eannot.1016.final_new		CHROMO superfamily domain-containing protein (transcription)	N	1.0	1.0	9.5			
HISTO_ZL.Contig1131.eannot.2109.final_new	*NIT100*	No BLAST hit; 38 aa	N	1.0	2.0	8.9			
HISTO_DA.Contig93.Fgenesh_histo.283.final_new		C6 finger transcription factor (transcription)	Y	1.0	1.5	8.1	1.1	1.0	1.3
HISTO_DM.Contig936.eannot.1251.final_new	*CPC1*	bZIP transcription factor CpcA, cross-pathway control protein (transcription)	Y	1.0	1.4	6.5	1.0	1.2	2.4
HISTO_DM.Contig933.Fgenesh_histo.156.final_new	*MSG5*	Tyrosine protein phosphatase MSG5	Y	1.7	1.0	6.1			
HISTO_DA.Contig93.Fgenesh_histo.126.final_new	*MSR1*	Arginyl-tRNA synthetase Msr1p	Y	1.0	1.0	5.7	1.2	1.0	1.9
HISTO_DA.Contig93.eannot.1958.final_new		Alternative oxidase (stress response)	Y	1.3	1.0	5.6	1.0	5.9	1.5
HISTO_LF.Contig359.eannot.2034.final_new	*PPS1*	Pps1 dual-specificity phosphatase	Y	1.7	1.0	5.4	1.0	2.9	4.7
HISTO_LF.Contig359.Fgenesh_Neurospora.151.final_new		cAMP-specific phosphodiesterase (cell signaling)	Y	1.0	1.7	5.4	2.2	1.3	1.0
HISTO_FX.Contig167.fgenesh_plus.6.final_new	*PNG1*	Peptidase PNG1	Y	1.0	1.3	5.2	1.0	1.1	1.5
HISTO_GL.Contig296.Fgenesh_histo.21.final_new		Copper fist DNA-binding domain-containing protein (transcription)	Y	1.0	1.1	4.9	1.0	1.1	1.4
HISTO_ZL.Contig1158.eannot.1400.final_new		Histidine kinase (protein phosphorylation—cell signaling)	Y	1.2	1.0	4.0			
HISTO_BP.Contig457.eannot.1770.final_new	*SSB1*	Heat shock protein SSB1	N	1.0	1.0	3.4			
HISTO_ZT.Contig181.Fgenesh_Neurospora.93.final_new	*ACO2*	Aconitate hydratase	Y	1.0	1.1	3.4			
HISTO_AL.Contig320.eannot.1088.final_new	*RPS28*	30S ribosomal protein S28e; 69 aa	N	1.0	1.1	3.3			
Selected G186AR genes enriched in conidia									
Contig22.4.eannot.1191.final	*NIT14*	Alcohol dehydrogenase; nitrosative stress-induced transcript	Y	3.8	1.0	1.7	27.8	1.0	2.3
Contig0.19.Fgenesh_Aspergillus.5.final	*ABC4*	ABC transporter	Y	14.2	1.0	9.6	18.2	1.0	4.9
Contig9.17-snap.4.final		C6 transcription factor (transcription)	Y	1.4	1.0	1.9	12.4	1.0	3.5
Contig8.21-snap.8.final		RNase III (RNA metabolism)	N				10.4	1.0	1.2
Contig0.29.Fgenesh_Aspergillus.28.final		C6 transcription factor (transcription)	Y	3.8	1.0	1.4	9.4	1.0	1.6
Contig15.15.eannot.1116.final		Similar to stress response RCI peptide (stress response)	Y	9.8	3.6	1.0	8.3	1.6	1.0

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TABLE 2 (Continued)

Systematic name	Gene name	Annotation	Ortholog detected[a]	Fold enrichment relative to lowest expression state[b]					
				G217B			G186AR		
				C	Y	M	C	Y	M
Contig5.26.Fgenesh_Aspergillus.10.final		C3HC4-type (RING finger) zinc finger-containing protein (transcription)	Y	10.6	1.0	20.9	8.3	1.0	2.7
Contig10.30.Fgenesh_histo.13.final		C6 zinc finger protein (transcription)	Y	2.4	1.0	1.2	8.0	1.3	1.0
Contig206.1.genewise.1.final		C2H2 transcription factor (AmdA) (transcription)	N				7.6	1.0	2.3
Contig3.42.Fgenesh_histo.2.final	*BRN2*	Laccase (brown-2) iron transport multicopper oxidase (pigment biosynthesis)	Y	4.6	1.0	3.6	7.5	1.0	1.8
Contig3.65.Fgenesh_histo.14.final	*NIT26*	Cytochrome P450 monooxygenase	Y	3.4	1.0	1.6	7.0	1.0	2.0
Contig3.11.Fgenesh_histo.10.final		Gal4 superfamily domain-containing protein (transcription)	Y	2.3	1.0	1.5	6.2	1.0	1.6
Contig0.54-snap.8.final	*DPS1*	Aspartyl-tRNA synthetase	Y	3.3	1.0	2.2	6.1	1.5	1.0
Contig3.40.Fgenesh_histo.5.final		C6 finger domain-containing protein (transcription)	Y	3.2	2.9	1.0	6.0	1.0	1.1
Contig0.3.Fgenesh_histo.11.final	*BMH1*	14-3-3-like protein	Y	1.1	1.4	1.0	6.0	1.0	1.2
Contig6.38.Fgenesh_Aspergillus.7.final		Phospholipase D (phospholipid catabolism)	Y				5.7	1.0	1.8
Contig9.3-snap.10.final	*WHC1*	GATA factor, white collar 1 (asexual development—transcription)	Y	3.3	1.0	1.7	5.2	1.0	1.3
Contig0.70.eannot.1009.final		Patatin-like phospholipase domain-containing protein (phospholipid catabolism)	Y	1.0	1.1	1.0	5.0	1.6	1.0
Contig18.4.eannot.1262.final		HMG box transcription factor, similar to MAT1-2 (transcription)	Y	3.2	1.0	22.9	4.9	1.0	1.6
Contig9.17.eannot.1073.final	*NIT60*	NEFA-interacting nuclear protein NIP30	Y	3.0	1.0	1.2	4.4	1.4	1.0
Contig9.30.Fgenesh_histo.15.final	*INV1*	Similar to involucrin repeat protein of *A. fumigatus* (cell wall organization)	Y	3.3	1.2	1.0	4.0	1.0	1.1
Contig0.92.Fgenesh_histo.10.final	*MON1*	Vacuolar fusion protein Mon1	Y	2.7	1.2	1.0	4.0	1.0	1.1
Contig10.1.Fgenesh_histo.6.final		Patatin-like phospholipase (phospholipid catabolism)	N				3.5	1.1	1.0
Contig20.8.Fgenesh_Aspergillus.5.final		NDT80/PhoG-like DNA-binding family protein (transcription)	Y	2.3	1.0	10.2	3.5	1.0	1.1
Selected G186AR genes enriched in yeast cells									
Contig18.2.Fgenesh_histo.12.final	*HPD1*	4-Hydroxyphenylpyruvate dioxygenase, HcHMM yeast-phase-induced protein	Y	1.0	1.0	1.0	3.3	12.5	1.0
Contig30.16.Fgenesh_histo.13.final	*GCL1*	Glutamate-cysteine ligase, yeast-phase-induced protein	Y	2.2	6.0	1.0	1.0	10.8	1.6
Contig2.27.Fgenesh_histo.3.final	*LAE1*	Methyltransferase LaeA (transcription)	Y	1.0	4.6	2.5	1.1	10.2	1.0
Contig24.6.Fgenesh_histo.9.final	*NIT22*	Peroxisomal dehydratase	Y	1.0	10.9	4.3	1.0	10.1	1.2

Contig16.4.genewise.3.final	*ZRT1*	Zinc/iron transporter	N				1.0	9.8	1.8
Contig26.8.fgenesh_plus.4.final	*IDH1*	Isocitrate dehydrogenase subunit 1	Y	1.0	4.1	2.1	1.0	9.0	1.5
Contig7.4.eannot.1208.final	*CCC1*	Calcium transporter CCC1, vacuolar iron transporter Ccc1	Y	4.5	12.7	1.0	2.7	8.9	1.0
Contig1.78-snap.3.final	*MTP1*	MFS phospholipid transporter, glycerophosphoinositol permease	Y	4.1	11.5	1.0	1.3	7.6	1.0
Contig3.39.fgenesh_plus.6.final	*AGS1*	Alpha-1,3-glucan synthase (cell wall organization)	Y				1.5	7.5	1.0
Contig14.7.Fgenesh_Aspergillus.3.final	*TRI7*	TRI7-like toxin biosynthesis protein	Y	1.1	1.5	1.0	1.0	7.4	2.4
Contig11.2.Fgenesh_histo.1.final	*FAT1*	AMP-dependent ligase, long-chain fatty acid transporter	Y	2.0	5.9	1.0	1.3	7.4	1.0
Contig30.15.Fgenesh_histo.13.final	*IDO1*	Indoleamine 2,3-dioxygenase (metabolism)	Y	1.0	1.6	1.5	1.0	6.8	1.9
Contig2.23-snap.11.final	*RED1*	Reductase RED1	Y				1.8	6.7	1.0
Contig3.2.Fgenesh_histo.5.final		AT DNA-binding protein (transcription)	Y	1.0	1.4	1.0	2.0	6.5	1.0
Contig14.4.eannot.1024.final	*NIT65*	Conserved protein of unknown function	Y	1.6	1.0	1.6	1.4	6.3	1.0
Contig0.92.eannot.1129.final		Alternative oxidase (stress response)	Y	1.3	1.0	5.6	1.0	5.9	1.5
Contig1.62.eannot.1131.final	*NIT59*	AN1-type zinc finger protein (protein interaction)	Y	1.0	1.5	2.0	1.0	5.5	1.2
Contig0.69.Fgenesh_Aspergillus.3.final	*YDJ1*	Mitochondrial import protein MAS5/Ydj1p	Y	1.0	2.4	5.8	1.0	5.3	1.3
Contig0.22.fgenesh_plus.6.final	*IDP1*	Isocitrate dehydrogenase Idp1	Y	1.0	2.2	1.2	1.6	5.3	1.0
Contig6.29.eannot.1009.final	*CLG1*	Cyclin-like protein (cell signaling)	Y				1.0	5.2	1.4
Contig12.3.eannot.1220.final	*MET3*	ATP sulfurylase, HMM yeast-phase-induced protein	Y	2.5	1.4	1.0	1.0	4.8	1.5
Contig9.30.eannot.1107.final	*AHA1*	Aha1 (Hsp90 cochaperone) domain family protein	Y				1.4	4.7	1.0
Contig14.6.Fgenesh_histo.31.final	*CHO1*	Choline sulfatase, *H. capsulatum* yeast-phase-induced protein	Y	2.7	7.0	1.0	1.0	4.6	1.4
Contig2.54.Fgenesh_Aspergillus.39.final	*NOP12*	Nucleolar protein 12, RNA-binding protein	Y	1.0	1.6	1.7	1.0	4.5	1.3
Contig0.108-snap.1.final	*GAC1*	Protein phosphatase regulatory subunit Gac1	Y	3.9	1.0	1.0	1.0	4.2	1.1
Contig20.9.eannot.1064.final	*SSC1*	Heat shock protein SSC1, Hsp70-like protein	Y	1.0	1.7	1.7	1.0	4.2	1.3
Contig9.15.Fgenesh_histo.11.final		C2H2 transcription factor (transcription)	Y	1.0	3.5	1.2	1.0	4.2	1.0
Contig12.2.eannot.1026.final		CHCH domain-containing protein (transcription)	Y	1.0	1.8	1.6	1.0	4.0	1.3
Contig11.14.fgenesh_plus.1.final		C2H2 transcription factor RfeC (transcription)	Y	1.0	2.0	4.4	1.0	3.9	1.0
Contig7.5.Fgenesh_histo.4.final	*RRF1*	Ribosome recycling factor	Y	1.5	2.5	1.0	1.0	3.6	1.1
Contig15.20.genewise.6.final	*OXA1*	Mitochondrial Oxa1p	Y	1.0	2.7	1.6	1.1	3.4	1.0
Contig11.27.eannot.1053.final	*XBP1*	APSES transcription factor (transcription)	Y	5.2	7.6	1.0	1.0	3.3	1.0

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TABLE 2 (Continued)

Systematic name	Gene name	Annotation	Ortholog detected[a]	Fold enrichment relative to lowest expression state[b]					
				G217B			G186AR		
				C	Y	M	C	Y	M
Selected G186AR genes enriched in mycelia									
Contig1.53.Fgenesh_Aspergillus.10.final		Fungus-specific transcription factor domain-containing protein (transcription)	Y	2.7	1.0	6.0	4.6	1.0	43.7
Contig91.3.eannot.1011.final		C6 transcription factor similar to AmyR (transcription)	Y	1.7	1.0	2.1	5.8	1.0	35.3
Contig29.8.Fgenesh_histo.5.final		Zinc finger protein (transcription)	Y	14.5	1.0	5.6	6.2	1.0	35.0
Contig22.5.eannot.1027.final	*NIT21*	*O*-Methyltransferase	Y	1.4	1.0	1.1	2.4	1.0	22.0
Contig8.24.Fgenesh_Aspergillus.13.final	*YPS3*	Yeast-phase-specific protein Yps-3	Y	1.9	22.1	1.0	7.2	1.0	21.8
Contig11.4.Fgenesh_histo.18.final		LaeA-like methyltransferase (transcription)	Y	2.5	1.0	1.8	5.0	1.0	18.1
Contig4.6.Fgenesh_Aspergillus.46.final	*SFG1*	SfgA negative regulator of *A. nidulans* conidiation (transcription)	Y	1.5	2.4	1.0	4.7	1.0	17.3
Contig10.5-snap.5.final		Zn(2)-Cys(6) binuclear cluster transcription factor (transcription)	N				4.1	1.0	16.3
Contig11.8.Fgenesh_Neurospora.4.final	*TYR3*	Tyrosinase (melanin biosynthesis)	Y	3.3	1.0	1.9	2.3	1.0	14.1
Contig29.6.genewise.1.final		C6 transcription factor (transcription)	N				1.5	1.0	13.8
Contig12.16-snap.29.final		Transcription factor RfeF (transcription)	N				2.9	1.0	12.4
Contig29.2.eannot.1010.final		C2H2 transcription factor (transcription)	N				1.6	1.0	11.9
Contig7.6.Fgenesh_histo.3.final		C6 finger domain-containing protein (transcription)	Y	5.7	1.0	7.5	2.9	1.0	11.1
Contig0.5.Fgenesh_histo.10.final		GAL4-like Zn(II)2Cys6 (or C6 zinc) binuclear cluster transcription factor (transcription)	Y	3.5	3.7	1.0	2.4	1.0	10.1
Contig13.3.Fgenesh_Aspergillus.27.final	*M46*	Mold-specific M46 protein (unknown function)	Y				1.1	1.0	9.5
Contig0.29-snap.16.final	*DPP4*	Extracellular dipeptidyl-peptidase Dpp4	Y	1.7	1.7	1.0	1.1	1.0	9.4
Contig14.7.Fgenesh_histo.28.final		NmrA family transcriptional regulator	Y	7.2	1.0	4.4	1.0	1.6	9.4
Contig3.3.Fgenesh_Aspergillus.6.final		C6 zinc finger domain-containing protein (transcription)	Y	1.1	1.2	1.0	1.8	1.0	8.4
Contig12.3.Fgenesh_histo.27.final	*SEC2*	GDP/GTP exchange factor Sec2p	Y	1.0	1.4	1.3	1.0	1.4	7.9
Contig2.66.Fgenesh_histo.2.final		bZIP transcription factor (transcription)	Y	1.9	1.0	4.5	1.9	1.0	7.4
Contig1.56.fgenesh_plus.5.final		C2H2 finger domain-containing protein (transcription)	Y	1.4	1.0	1.0	2.1	1.0	7.1
Contig7.12.eannot.1016.final	*RDI1*	Rho-GDP dissociation inhibitor	Y	1.0	1.0	1.8	1.2	1.0	5.9
Contig16.3.Fgenesh_histo.2.final		Fungus-specific transcription factor (transcription)	Y				1.2	1.0	5.4
Contig35.4.Fgenesh_histo.1.final	*SCY1*	Protein kinase Scy1, clathrin-coated vesicle protein	Y	1.8	1.3	1.0	1.2	1.0	5.2
Contig11.20.eannot.1047.final	*SIT1*	Siderochrome-iron uptake transporter Sit1	Y	3.1	1.0	2.4	1.2	1.0	5.0
Contig231.1.fgenesh_plus.1.final	*SEB1*	C2H2 transcription factor (transcription)	Y	1.0	1.0	2.8	1.5	1.0	5.0

Contig25.2.genewise.6.final	*DPP5*	Secreted dipeptidyl peptidase DppV	Y	1.9	1.0	2.4	1.4	1.0	4.9
Contig0.13.Fgenesh_histo.10.final	*TYR2*	Tyrosinase (melanin biosynthesis)	Y				1.0	1.0	4.7
Contig4.14.eannot.1038.final	*FNX1*	Multidrug resistance protein Fnx1	Y	1.0	3.1	1.0	1.2	1.0	4.1
Contig1.40.eannot.1029.final	*GLS1*	1,3-Beta-glucan synthase component GLS1	Y	1.0	2.6	4.6	1.1	1.0	3.9
Contig3.6.eannot.1095.final	*MKC1*	Mitogen-activated protein kinase MKC1 (protein phosphorylation)	Y	1.0	1.6	1.9	1.0	1.3	3.9
Contig28.6.eannot.1011.final		C6 zinc finger domain-containing protein (transcription)	Y				1.1	1.0	3.7
Contig31.4.Fgenesh_Neurospora.6.final	*APS1*	Anucleate primary sterigmata protein ApsA (asexual development)	Y	1.9	1.0	4.4	1.0	1.0	3.1

[a] Y, ortholog detected; N, no orthologs detected.
[b] C, conidia; Y, yeast cells; M, mycelia.

main that is highly conserved among filamentous fungi and thermal dimorphs such as *H. capsulatum* (40, 52). The founding velvet family member, VeA, functions in a complex with LaeA, a transcriptional regulator, and VelB to regulate development and secondary metabolism in filamentous fungi (53–55; for a review, see reference 52). The precise function of VelC in *A. nidulans* remains obscure, although deletion of *velC* results in a slight increase in sexual fruiting body formation (53) and a reduction in conidiation (40). Although the *H. capsulatum* VosA ortholog (Ryp2) and VelB ortholog (Ryp3) are upregulated in the yeast form of G217B and required for yeast-phase growth (56), the *VELC* transcript was highly enriched in mycelial cells of both strains (Table 1). We observed enriched expression in yeast for the velvet family protein Ryp2 in our G217B and G186AR data set (Fig. 4B and C), although the fold enrichment in G186AR did not meet our threshold criteria.

H. capsulatum is a heterothallic fungus. For mating to occur, one mating partner must contain the *MAT1-1* (+) mating type locus, which is present in G217B and encodes an α-box transcription factor, and the other partner must bear the *MAT1-2* (−) mating type locus, which is present in G186AR and encodes an HMG domain transcription factor (57, 58). *H. capsulatum* is known to mate in the filamentous form (59), and as expected, we observed mycelial enriched expression of *MAT1-1* in the G217B strain and a moderate enrichment of *MAT1-2* (2.30-fold) in mycelia (relative to yeast) in the G186AR strain.

Our transcriptomic and annotation analyses uncovered phase-specific enriched expression of the majority of the tyrosinase protein family. Tyrosinases, also known as polyphenol oxidases, are enzymes that can be used to produce melanins and other products in cells (60). In *N. crassa*, tyrosinase expression is induced during starvation, during sexual development (61), or by the addition of exogenous cyclic AMP (cAMP) (62). In the filamentous bacterium *Streptomyces lividans*, ectopic expression of the tyrosinase MelC induces precocious development of aerial mycelia, suggesting a positive role for tyrosinases in morphological development (63). We identified a gene family of seven putative tyrosinases (*TYR1* to *TYR7*) in the *H. capsulatum* genome and examined their differential expression (Fig. 6). *TYR1*, *TYR2*, and *TYR6* showed mycelial enriched expression, although data for *TYR2* were missing for G217B and data for *TYR6* were missing for G186AR. *TYR4* and *TYR5* were expressed in mycelia and conidia but depleted in yeast, and *TYR3* showed mycelial enrichment in G186AR and some conidial enrichment in G217B. Phase-specific enriched expression of GFP driven by the *TYR1* promoter is shown in Fig. 5. Notably, none of the tyrosinase genes showed enriched expression in yeast cells, suggesting that they play a role in the biology of mycelia and conidia.

In addition to protein-encoding genes, probes for tRNA transcripts were present on the G217B microarray. We found that the tRNA transcripts, with the exception of tRNA Met-(CAT) and tRNA Leu-(CAA), showed their highest levels of expression in mycelia and were generally depleted in conidia (Fig. 7). In *A. fumigatus*, conidiation induces tRNA depletion, which has been proposed to result in downregulation of protein synthesis in vegetative spores (64).

Yeast-specific enriched transcripts encode siderophore biosynthesis genes, secreted proteins, catalases, and transcription factors. We determined that 175 transcripts were preferentially expressed in G217B yeast cells and 159 transcripts were preferentially expressed in G186AR yeast cells. Forty-five of the corre-

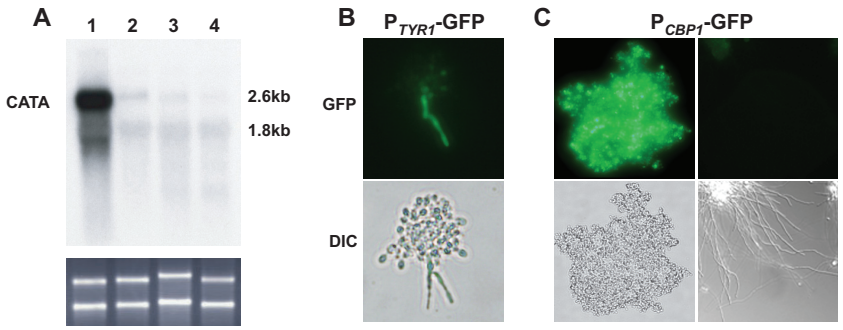

FIG 5 Validation of phase-specific enriched expression. (A) Northern blot analysis of *CATA* expression was performed on total RNAs isolated from G217B conidia (lane 1), yeast (lane 2), and mycelia (shaking culture [lane 3] or still culture [lane 4]). Ethidium bromide visualization of rRNAs from the same samples is shown below the blot as a loading control. Fluorescence microscopy was used to examine strains expressing GFP under the control of either the *TYR1* promoter (B) or the *CBP1* promoter (C). In panel B, GFP fluorescence was monitored in cells that were undergoing filamentation at room temperature. GFP expression is observed at much higher levels in a filamentous cell than in yeast-form cells, consistent with mycelial enriched expression. In panel C, GFP fluorescence is observed only in the clump of yeast-phase G186AR cells (left), not in G186AR cells undergoing filamentation at room temperature (right), consistent with yeast-specific enriched expression. DIC, differential interference contrast.

sponding genes showed yeast-specific expression in both *H. capsulatum* strains (Fig. 4D and Table 1). In contrast to mycelia and conidia, yeast cells expressed a smaller number of highly phase-specific transcripts, most of which have orthologous genes in other species (Table 1).

Yeast-specific enriched transcripts included those previously characterized in G217B as encoding an iron-regulated siderophore biosynthetic gene cluster including the *ABC1*, *SID1*, *SID3*, *SID4*, and *OXR1* genes (16). Here we observed that yeast-specific expression of the siderophore biosynthetic gene cluster was conserved in the G186AR strain (Table 1). Notably, G217B *SID1* is required for normal colonization of tissues in the mouse model of histoplasmosis (16).

H. capsulatum possesses two predicted catalases in addition to the CatA transcript enriched in conidia as described above. Catalase B (*CATB*), also known as the M antigen (65, 66), is a yeast-phase-specific protein of *H. capsulatum*. The CatB protein was recently demonstrated to be secreted by *H. capsulatum* yeast-phase cells (67). Our analysis indicates that *CATB* is part of a core, conserved set of yeast-phase-specific genes of *H. capsulatum*, as is the other yeast catalase gene, *CATP*.

CBP1, a yeast-phase-specific gene and virulence determinant of *H. capsulatum* (68), was identified as a G217B yeast-specific transcript in our data set. In G186AR, although *CBP1* has previously been shown to have high differential expression in yeast cells (69), the fold enrichment in yeast over mycelia in our data set did not meet our criteria, causing the *CBP1* gene to drop out of the conserved, yeast-specific gene set and to be included only in the strain-specific gene set. Nonetheless, we confirmed that we observed yeast-specific enriched expression of GFP driven by the *CBP1* promoter in the G186AR strain, as shown in Fig. 5. Other previously identified strain-specific yeast enriched transcripts included *AGS1*, which encodes an alpha-1,3-glucan synthase that is known to be highly expressed in G186AR but not in G217B due to a promoter insertion (10). Additionally, as previously described (6), the *YPS3* (yeast-phase-specific gene 3) gene showed yeast-specific expression in G217B but mycelium-specific expression in G186AR (Table 2). Finally, we also observed that *YPS21* (yeast-phase-specific gene 21), a previously identified yeast-specific gene in *H. capsulatum* (70), was enriched in yeast in both G217B and G186AR.

LDF1 is a gene encoding a protein of unknown molecular function that is required for optimal macrophage lysis by the G217B strain *in vitro* (D. T. Isaac and A. Sil, unpublished observations). *LDF1* was the most highly differential yeast-specific gene in the G217B set, showing a relative differential expression of 96-fold (Table 2). No *LDF1* expression data were available for the G186AR strain. In addition, two genes of unknown function (Contig37.5.eannot.1055.final and Contig8.3.eannot.1103.final) had extraordinarily high levels of differential expression in yeast cells (77- and 90-fold, respectively, in G186AR and approximately 45-fold for each gene in G217B) (Table 1). Whereas the functions of these genes are unknown, their high levels of differential expression suggest that they may play important roles in yeast-phase biology.

Yeast cells highly express a transcript, *LYP1*, predicted to encode a membrane transporter that may facilitate cystine transport. Cystine is composed of two covalently attached cysteine molecules, and *H. capsulatum* yeasts require cysteine supplementation for *in vitro* growth. Although *LYP1* was originally identified as a putative yeast-phase-specific lysine permease gene (12), we reannotated it *CYN1* here, since its *C. glabrata* ortholog was recently shown to function in cystine transport (71).

Several putative transcriptional regulators showed yeast-specific expression. For example, an ortholog of the *A. nidulans* transcription factor FacB displayed highly enriched expression in yeast cells of both strains. The corresponding *H. capsulatum* factor was recently shown to be required for yeast-phase growth in the G217B strain and thus has been named Ryp4 (required for yeast-phase growth 4) (S. Beyhan, M. Gutierrez, M. Voorhies, A. Sil, submitted for publication). Other transcription factors displaying yeast-specific enriched expression included *H. capsulatum* Cph1, an ortholog of the Ste12 transcriptional regulator of sexual development and morphology in *S. cerevisiae*. Additionally, yeast-specific enriched expression was displayed by the *H. capsulatum* ortholog of Xbp1, a transcriptional repressor in *S. cerevisiae* (72). Finally, Lae1, the *H. capsulatum* ortholog of LaeA, a putative methyltransferase involved in regulation of secondary metabolism biosynthetic gene expression in *A. nidulans* (53, 54), showed yeast-specific enriched expression. Interestingly, the corresponding *A. fumigatus* ortholog of Lae1 is required for resistance to attack by neutrophils (73).

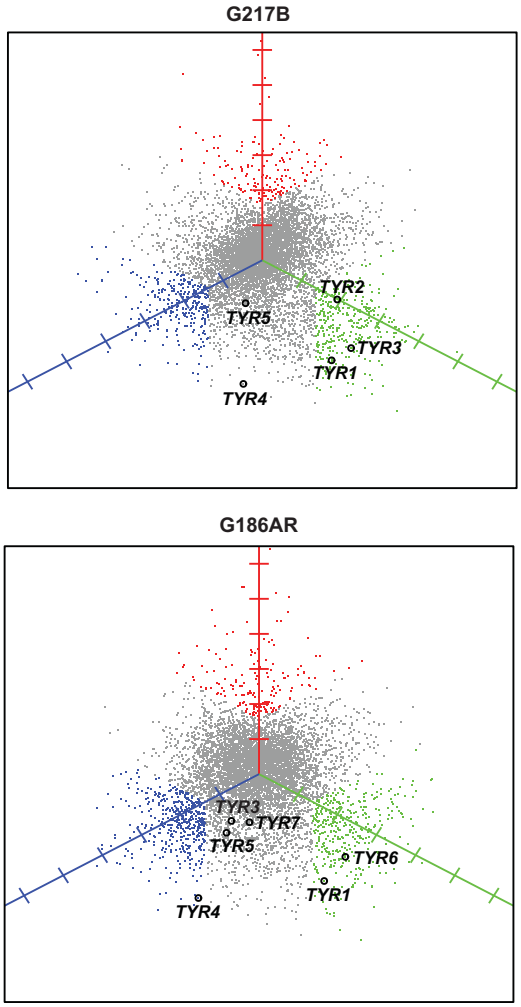

FIG 6 The tyrosinase gene family shows phase-specific expression. Relative enrichment plots are shown for G217B (A) and G186AR (B). Yeast, mycelial, and conidial axes are drawn in red, green, and blue, respectively; axis ticks indicate $\log_2$ units of enrichment. Genes were plotted by projecting the BAGEL-estimated relative expression values on the corresponding axes (the condition of lowest expression always has a $\log_2$ enrichment value of zero). Yeast, mycelial, and conidial enriched genes, based on the 3-fold enrichment criterion, are colored red, green, and blue, respectively. Tyrosinases are highlighted with black circles and labeled.

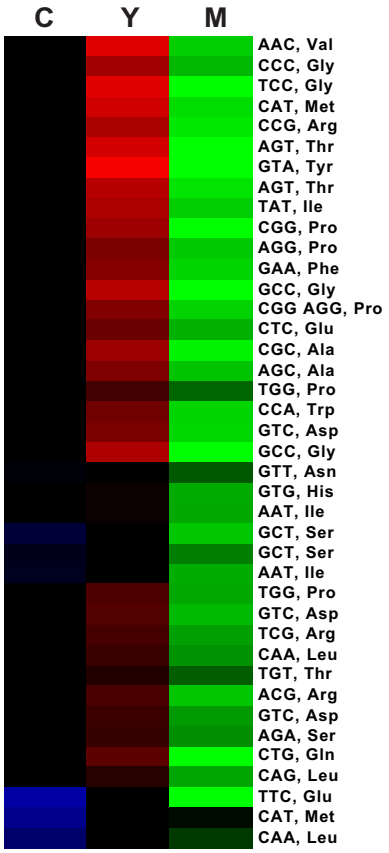

FIG 7 tRNAs are differentially expressed in conidia, yeast, and mycelia. The heat map shows the enriched expression in mycelia and depleted expression in conidia of 40 tRNA transcripts of G217B. Relative transcript levels in conidia (C), mycelia (M), and yeast (Y) are displayed. Intensities are $\log_2$ BAGEL-estimated relative expression levels from 0 (black) to 4 (saturated). tRNAs are labeled with cognate amino acids and anticodons as predicted by tRNAscan-SE.

In sum, conidia and mycelia constitute the infectious phases of *H. capsulatum*, and microconidia are thought to be the primary infectious particles encountered by a host in nature, due to their small size. This is the first study of a thermally dimorphic fungal pathogen where viable, infectious conidia were purified and subjected to whole-genome transcriptomic analysis. Since yeast and mycelial cells were profiled in parallel, this analysis provides a rich data set that gives a molecular fingerprint of each developmental state. Furthermore, by performing these studies with two highly divergent strains of *H. capsulatum*, we were able to identify core genes that show evolutionarily conserved phase-specific expression. Future studies will mine these data to characterize novel factors for their roles in the infectious and parasitic phases of *H. capsulatum*. Additionally, these studies raise interesting questions about when and where conidial enriched transcripts are synthesized during the development of vegetative spores—for example, are conidial enriched transcripts synthesized in filamentous cells and then packaged into spores by unknown mechanisms? Finally, the manual and computational annotation provided here, coupled with phase-specific expression data for each predicted gene in two *H. capsulatum* strains, constitutes a valuable community resource for further molecular and genomic analyses of *H. capsulatum* and other thermally dimorphic fungal pathogens.

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