Transcriptional Reprogramming of the Mycoparasitic Fungus *Ampelomyces quisqualis* During the Powdery Mildew Host-Induced Germination

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ABSTRACT

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Ampelomyces quisqualis is a mycoparasite of a diverse range of phytopathogenic fungi associated with the powdery mildew disease. Among them are several *Erysiphaceae* species with great economic impact on high-value crops such as grape. Due to its ability to parasitize and prevent the spread of powdery mildews, *A. quisqualis* has received considerable attention for its biocontrol potential. However, and in sharp contrast to the extensively studied biocontrol species belonging to the genus *Trichoderma*, little is known about the biology of *A. quisqualis* at the molecular and genetic levels. We present the first genome-wide transcription profiling in *A. quisqualis* during host-induced germination. A total of 1,536 putative genes showed significant changes in transcription during the germination of *A. quisqualis*. This finding denotes an extensive transcriptional reprogramming of *A. quisqualis* induced by the presence of the host. Several upregulated genes were predicted to encode for putative mycoparasitism-related proteins such as secreted proteases, virulence factors, and proteins related to toxin biosynthesis. Our data provide the most comprehensive sequence resource currently available for *A. quisqualis* in addition to offering valuable insights into the biology of *A. quisqualis* and its mycoparasitic lifestyle. Eventually, this may improve the biocontrol capacity of this mycoparasite.

Additional keywords: gene expression profiling, transcriptome.

Ampelomyces quisqualis is a naturally occurring hyperparasite of the family Erysiphaceae, the cause of powdery mildews on various economically important crops (3,39–41). Although *A. quisqualis* is specific to powdery mildews, it has a broad host range within this group of fungi. It has been found to be associated with more than 60 species from eight different genera of the order Erysiphales (41). The biology and life cycle of *A. quisqualis* have been extensively studied and have been reviewed previously (40,41). In brief, conidia of *A. quisqualis* are produced in pycnidia, which are developed intracellularly in the parasitized mycelia of the powdery mildew host. Conidia are released in the presence of water and, under suitable conditions, can germinate and the hyphae of *A. quisqualis* can then penetrate the nearby hyphae of powdery mildew. During the winter, *A. quisqualis* is able to survive in the form of pycnidia produced saprotrophically in dead plant tissues but it is not considered a good saprophyte. *A. quisqualis* is able to infect and form pycnidia within powdery mildew hyphae, conidiophores, and chasmothecia (41). This results in the reduction of growth and eventually the death of the parasitized mildew. Because of its parasitic properties, *A. quisqualis* has been identified as a promising biocontrol agent against several species of powdery mildew (39,41,79). A strain of *A. quisqualis* was the first biofungicide (AQ 10, marketed by CBC Europe, Italy) ever developed for powdery mildew control. A few other isolates are also available on the market currently (Q-fect; Green Biotech, Korea; and POWDERYCARE; AgriLife, India).

The anatomical and some physiological aspects of mycoparasitic activity in *A. quisqualis* have been widely investigated (2,4, 33,40,41). Although conidia of *A. quisqualis* germinate poorly in water or in the presence of glucose, studies suggest that the presence of an as-yet-unknown water-soluble substance from the host is able to trigger its germination (31,80). After germination, *A. quisqualis* is able to penetrate and parasitize host hyphae, probably through mechanical (81) or enzymatic processes (66). The isolation of an *exo*-β-1,3-glucanase from *A. quisqualis* has already been reported and its expression has been studied both in vitro and in vivo (66). Furthermore, in vitro production of lytic enzymes has been reported within different isolates of *A. quisqualis,* suggesting a possible role in the mycoparasitic process (4). However, the molecular pathways involved in the mycoparasitism of *A. quisqualis* are largely unknown. Moreover, no genomic resources allowing analysis of the traits of this mycoparasitic interaction are available as yet.

Over the last few years, expressed sequence tag (EST) libraries have been extensively used in large-scale transcriptional studies to explore molecular events during different types of mycoparasitic interactions (46,56,58,71). Advances in genomics and transcriptomics have also provided new opportunities for improving our understanding of the mycoparasitic interactions (5,42,64). High-throughput 454 pyrosequencing was first used to boost the identification and quantification of transcripts in model species

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for which genomic or extensive Sanger EST data can provide an assembly reference for the 454 reads (18,78). Recently, this approach has also been successfully used to build up an extensive catalogue of unique transcripts in organisms for which the genome sequence is not available (6).

In this study, a high-throughput sequencing approach was adopted to produce a representative transcript catalogue for the nonmodel species of *A. quisqualis* during mycoparasitic interactions with *Podosphaera xanthii*. This catalogue was then used to build up an oligonucleotide microarray for large-scale analysis of transcriptional changes occurring during the early germination phase of *A. quisqualis*.

MATERIALS AND METHODS

Sample preparation for different mycoparasitism stages. The system studied was made up of the mycoparasite *A. quisqualis* strain CNCM I-807 (Collection Nationale de Cultures de Microorganismes, Paris), the fungal host *P. xanthii* (collected from *Cucurbita pepo* plants in northern Italy in August 2007), and plants of *C. pepo* 'Xsara'. *A. quisqualis* was grown on potato dextrose agar (Oxoid, UK) at 25°C. *P. xanthii* was maintained on *C. pepo* plants, which were grown in peat in 1-liter pots in controlled greenhouse conditions (22 ± 3°C, 70 ± 10% relative humidity [RH], and ambient photoperiod).

A. quisqualis conidia were collected as described by Gu and Ko (31). Conidial suspensions were adjusted to a final concentration of 4×10^6 conidia/ml. *P. xanthii* conidia and mycelia were vacuumed from heavily infected leaves with a microvacuum system and suspended in sterile distilled water at a final concentration of 1×10^4 conidia/ml. For sample preparation, the mycoparasitic process was divided into three stages: host recognition (induction of the germination of *A. quisqualis* conidia in the presence of *P. xanthii* conidia), early parasitism (the formation of appressorium-like structures), and late parasitism (formation of mature pycnidia). Material for the host-recognition stage was prepared by mixing equal volumes of conidial suspensions containing the aforementioned concentrations of both *A. quisqualis* and *P. xanthii* and incubating for 24 h at 25°C. The higher concentration of *A. quisqualis* conidia relative to *P. xanthii* conidia was chosen to enrich our sample for *A. quisqualis* transcripts and facilitate downstream analysis. Then, the conidia (50 mg) were collected by filtration on Purabind 08 filter paper (Whatmann, Dassel, Germany), frozen in liquid nitrogen, and lyophilized (Heto LyoLab 3000; Thermo Scientific Heraeus, Loughborough, UK). For the early and late stages of parasitism, 10 ml of *P. xanthii* conidial suspension (1×10^4 conidia/ml) were sprayed onto 30 plants that each had five true leaves. Plants were left to dry and then incubated overnight at 22°C and high RH (95 to 98%). When powdery mildew symptoms first appeared (14 days after inoculation), 15 of the plants were treated with 10 ml of *A. quisqualis* suspension (3.5×10^6 conidia/ml; 10 ml/plant). Parasitism was monitored daily using a stereomicroscope (Hund Wetzlay H 600 LL, Wetzlar, Germany) and a light microscope (Nikon SMZ 800, Tokyo) (Supplementary Figure 1). At 2 and 14 days after treatment (early and late parasitism stage, respectively), *A. quisqualis*-parasitized powdery mildew (100 mg) was sucked into 2-ml sterile micro test tubes (100 mg/tube) using a vacuum system, frozen in liquid nitrogen, and lyophilized. Three independent experiments were carried out at each stage of the mycoparasitic relationship.

Quantification of relative RNA abundance in different mycoparasitism samples. Total RNA was extracted from lyophilized material from the host-recognition and early and late parasitism stages using the SV total RNA extraction kit (Promega, Milan, Italy) according to the manufacturer's instructions. The presence of RNA was assessed by agarose gel electrophoresis according to Sambrook et al. (69) and the quality and quantity of the RNA in the samples was evaluated with NanoDrop (Thermo Scientific, Wilmington, DE). To determine the relative abundance of *A. quisqualis*, *P. xanthii*, and *C. pepo* transcripts, the RNAs of the three organisms were quantified with real-time reverse-transcription polymerase chain reaction (RT-PCR) using specific primer pairs based on the 28S transcribed region using Primer3 software (77) available online (http://frodo.wi.mit.edu). The primer sequences were *A. quisqualis* forward: 5′-TCTCTTTG GGGAGGCCTTAT-3′; *A. quisqualis* reverse: 5′-GGCTTGACAC CCAAACACTC-3′; *P. xanthii* forward: 5′-GGTGGAATGTGG CTGTCTTT-3′; *P. xanthii* reverse: 5′-CACCCAAACACTCG CATAGA-3′; *C. pepo* forward: 5′-TTGTTACGCTTGTGGA GACG-3′; and *C. pepo* reverse: 5′-CGCACACATGTCAGACTC CT-3′. Primer specificity was previously tested on the genomic DNA extracted with the Nucleo Spin Plant kit (Macherey Nagel, Duren, Germany). Real-time RT-PCR was then performed on cDNA from the three stages to quantify the proportion of the RNA of each organism in the samples. cDNA was synthesized with RT-PCR using Ready-To-Go T-Primed First-Strand Kit (GE Healthcare Europe, Paris, France). Reactions were performed using Platinum SYBR Green quantitative PCR (qPCR) SuperMix-UDG kit (Invitrogen, Carlsbad, CA) in a 25-µl reaction volume in an MX 3000P Stratagene real-time PCR machine (Stratagene, La Jolla, CA).

cDNA library sequencing. Samples containing the largest proportions of mycoparasite RNA for each mycoparasitism stage were used for subsequent high-throughput sequencing analysis. Samples obtained from the host-recognition and early-parasitism stages were pooled together, and the same quantity of *A. quisqualis* total RNA was added for each of the two stages, to obtain a sample containing 0.2 µg of final RNA content. A second sample was made up of RNA purified from the late parasitism stage (0.2 µg). cDNA normalized libraries were prepared by Eurofins MWG Operon, Ebersberg, Germany (www.eurofinsdna.com) using the protocol described by Bellin et al. (6) (Supplementary File 1). Libraries were sequenced using GS FLX technology (Roche Diagnostics, Branford, CT) following standard protocols (51).

De novo assembly. The 454 reads were quality filtered according to standard parameters and cleaned from adaptor sequences. Sequences from each library were de novo assembled into contigs as described by Bellin et al. (6), using Newbler v1.1 (Roche 454) with a minimum reads overlap length of 40 bp, a minimum overlapping match between reads of 90%, a seed length of 16 bp, and a seed step of 12 bp. Redundancy between the contigs obtained from the two assemblies was removed by clustering sequences with an identity threshold of 85% and a coverage threshold of 85%; the longest sequence in the cluster was retained as cluster representative sequence.

Microarray construction. Oligonucleotide probes between 35 and 40 bp in length were designed from the 454 unigene sequences using OligoArray 2.1 software (67), as described by Bellin et al. (6). Custom 90K CombiMatrix arrays (AmpeloArray 1.0) were prepared with the oligonucleotide sequences designed, based on the 454-derived unigenes using a Custom Array Synthesizer (CombiMatrix, Mulkiteo, USA). DNA was extracted from *A. quisqualis* conidia using GeneElute Plant Miniprep Kit (Sigma-Aldrich) according to manufacturer's instructions. DNA (5 µg) was labeled using the ULS labeling kit with Cy5 for Combimatrix arrays (Kreatech Diagnostics, The Netherlands) according to the manufacturer's instructions. Prehybridization, hybridization, washing, and imaging were performed according to the manufacturer's protocols. Scanning was performed on a GenePix 4400A scanner. Parameters were set in order to have $1E^{-6}$ normalized counts at the 65,000 intensity level (saturation). Data extraction was carried out using CombiMatrix Microarray Imager software and a quantile normalization of data was performed. Selection of validated probes was performed by setting an intensity threshold equal to the mean plus two standard deviations

of the negative reference oligos (9). Custom 90K CombiMatrix arrays (AmpeloArray 2.0) were prepared with the oligonucleotide sequences validated using a Custom Array Synthesizer according to manufacture instructions (CombiMatrix).

Experimental design and microarray hybridization. To study the changes in gene expression during the host-recognition phase, a dialysis set-up was developed to stimulate in vitro germination of *A. quisqualis* conidia, while avoiding any contamination with *P. xanthii* and *C. pepo*. A 12,000-Da molecular weight cut-off dialysis sack (Sigma, Milano, Italy) was filled with *C. pepo* leaf disks (60 discs, 5 mm in diameter each) infected with *P. xanthii* and immersed in an aqueous suspension (500 ml) of *A. quisqualis* at 5×10^5 conidia/ml at room temperature. To maintain homogenous conditions, the system was stirred constantly. Equal amounts of conidia (7.5×10^7 conidia for each collection point) were collected 3, 6, 12, 24, 48, and 72 h after immersion. These samples were pooled into two groups. Those collected at 3, 6, and 12 h after immersion were pooled together in an "early-germination" group and those sampled at 24, 48, and 72 h after immersion were pooled together in a "late-germination" (visible germ tubes) group. Total RNA was extracted from the two samples representing the two recognition phases, as well as dormant conidia directly collected from *A. quisqualis* cultures on petri dishes (0 h). Extraction was performed using the Sigma Spectrum Plant Total RNA kit (Sigma). Reverse transcription, amplification, and labeling were performed with the RNA AmpULSe amplification and labeling kit according to the manufacturer's instructions (Kreatech Diagnostics). Prehybridization, hybridization, washing, and imaging were performed according to the manufacturer's protocols. Three biological replicates were analyzed for each condition. Scanning was performed on GenePix 4400A scanner (Molecular Devices, Sunnyvale, CA).

Microarray data analysis. Microarray raw data were extracted using CombiMatrix Microarray Imager software and quantile normalization of data was performed using CombiMatrix 6.0 software. Differentially expressed transcripts were identified using linear model analysis, as implemented in Limma (75). These were considered significant when the log2 fold change was ≥1 or ≤–1 and the adjusted *P* value was ≤ 0.05. A sequence was considered to be expressed when the signal intensity of its corresponding probe was above a threshold level, calculated as the mean plus two times the standard deviation of the negative reference samples included in the array design (9). To increase the power of subsequent statistical tests, nonexpressed genes were removed from the data set. Clustering was performed using Tiger TMEV 4.1 software on differentially expressed genes to identify regulated groups of genes. K-means mode, in which similarity in the expression patterns of different genes is measured in terms of Pearson's correlation coefficients, was used. Functional annotation of the transcripts was carried out using the Uniprot protein database of Swiss-Prot and TrEMBL (11) using the BLASTX algorithm with an e-value cut-off of 1×10^{-6}. Further functional characterization and enrichment analysis was performed by assigning gene ontology (GO) terms to the sequences using the Blast2GO program (20). All microarray expression data are available at the Gene Expression Omnibus (27) under the series entry GSE22888.

qRT-PCR analysis. All primers used for the qRT-PCR experiments were designed using Primer 3 software (77) on the basis of the probe sequences present on the array. Primer sequences are listed in Supplementary Table 1. First-strand cDNA was obtained for each of the three biological replicates using Superscript III (Invitrogen) and an oligo-dT primer according to standard protocols described by the manufacturer. The qPCR reactions were performed in triplicate (technical replicates) with Platinum SYBR Green qPCR SuperMix-UDG (Invitrogen) using Light Cycler 480 (Roche Diagnostic, Mannheim, Germany). The qPCR conditions were 50°C for 2 min and 95°C for 2 min as initial steps, followed by 40 cycles at 95°C for 15 s and then 60°C for 1 min. Dissociation curves were analyzed to verify the specificity of each amplification reaction. Light Cycler 480 SV1.5.0 software (Roche) was used to calculate cycle threshold (CT) values using the second derivative calculation (48). The relative expression ratio value for each gene was calculated relative to the elongation factor 1-α, which was chosen for normalization purposes because its expression was not affected during conidial germination, using the following formula: relative expression ratio = $2^{Ct_{\text{target gene}} - Ct_{\text{reference gene}}}$.

Accession numbers. Transcriptome sequence data from this article are available in the National Center for Biotechnology Information (NCBI) Sequence Read Archive under accession number SRP002892. Microarray data reported in this article are available from NCBI Gene Expression Omnibus database (27) under accession number GSE22888.

RESULTS

Evaluation of the relative abundance of *A. quisqualis* RNA during the mycoparasitic process. The relative abundance of RNA from *A. quisqualis* and the powdery mildew host *P. xanthii* growing on *C. pepo* was determined with qRT-PCR, using primers specific for the 28S ribosomal RNA region of the three organisms. Total RNA was extracted from three stages of the mycoparasitic process: host-recognition (*A. quisqualis* and *P. xanthii* conidia in water) and early and late parasitism phases (*A. quisqualis* and *P. xanthii* on *C. pepo*), corresponding to 2 and 14 days, respectively, after treatment of the powdery-mildew-infected plant with *A. quisqualis*. As expected in the host-recognition stage, *A. quisqualis* RNA was predominant (95 ± 4% standard error; average of three independent extractions), due to the 100-fold higher concentration of *A. quisqualis* conidia (see Materials and Methods section) (Supplementary Figure 2). In the early parasitism stage, RNA of *A. quisqualis* and RNA from *P. xanthii* were present in almost the same proportion (47 ± 2 and 50 ± 3%, respectively). However, in the late stage of parasitism, *A. quisqualis* RNA was far more prevalent (83 ± 7%) than that of *P. xanthii* RNA (15 ± 5%). In the two parasitism stages, the RNA included a relatively small amount of *C. pepo* RNA (2 ± 1%). Therefore, we considered that the relative abundance of *A. quisqualis* mRNAs was large enough to produce a transcript catalogue in which the *A. quisqualis* transcriptome was sufficiently represented.

Generation of a representative *A. quisqualis* transcript catalogue during mycoparasitism and expression microarray design. A high-throughput sequencing approach was utilized in order to produce a representative transcript catalogue during the interaction of *A. quisqualis* with its powdery mildew host (*P. xanthii*). Normalized cDNA libraries were obtained from two samples representing different stages of mycoparasitism process (i.e., combined host-recognition and early parasitism phase and late parasitism phase) and sequenced using 454 pyrosequencing, as previously described (6). An overview of the experimental workflow is presented in Supplementary Figure 3 while sequencing and assembly data are summarized in Table 1. Overall, the data obtained from the two libraries yielded a total of 62,636 nonredundant sequences. A microarray was then constructed using a set of 59,611 specific nonredundant oligonucleotide sequences and validated by hybridization of *A. quisqualis* genomic DNA. A subset of 31,222 probes whose signal intensity was above the mean plus two standard deviations of the negative reference oligos (9) was finally used to construct an expression microarray to study the transcriptional reprogramming of *A. quisqualis* during germination.

The 31,222 *A. quisqualis* sequences were functionally annotated based on sequence similarity searches against UniProt (SwissProt and TrEMBL) databases (83) using the BLASTX algorithm and an e-value cut-off of 1×10^{-6}. In total, 16,021 (51.3%) of the *A. quisqualis* sequences had at least a significant hit, with 7,214 sequences having a significant match to known

proteins in SwissProt while 8,807 sequences gave a significant match in TrEMBL (Supplementary Table 2). The remaining 15,201 (48.7%) sequences had no match to any of the databases and were classified as transcripts of unknown function. Almost 40% of the sequences had matches with the Ascomycetes fungi *Phaeosphaeria nodorum,* followed by *Pyrenophora teres* f. *teres* (11%) and *Leptosphaeria maculans* (9%) (Supplementary Figure 5).

General transcriptional response and analysis of expression profiling during *A. quisqualis* germination phases. Total RNA was obtained from *A. quisqualis* dormant conidia as well as in the early and late germination phase (0 h, 3 to 12 h, and 24 to 72 h poststimulation, respectively) and used for microarray hybridization (see Materials and Methods). Considering a false discovery rate (FDR) ≤ 0.05 (8) and an absolute value of log2 ratio ≥1, 1,536 transcripts were found to be differentially regulated during *A. quisqualis* germination in response to the powdery mildew host (Supplementary Table 3). Interestingly, we observed rapid and extensive transcriptional reprogramming in the early stages of *A. quisqualis* host-induced germination. Indeed, the expression of 812 transcripts, almost a third of differentially regulated transcripts, was modulated (473 upregulated and 339 downregulated) during the early germination phase (Fig. 1A).

The 1,536 differentially regulated genes were subjected to cluster analysis using TIGR MultiExperiment Viewer software (68). Genes were divided into six representative clusters, each of which had distinctive expression profiles (Fig. 1B; Supplementary Figure 5). Cluster I contains 438 genes that were overexpressed during the germination process and which were already upregulated during the early phase of the germination. Cluster II groups 175 genes that showed maximum expression levels in the early germination phase, while cluster III groups 287 genes that showed maximum expression levels during the late germination phase. Cluster IV consists of a subset of 52 genes for which expression was downregulated transiently during the early phase of *A. quisqualis* germination, then increased during the later phase. Finally, clusters V and VI contain 204 and 380 genes, respectively, showing maximum expression levels in dormant conidia and which were downregulated over the course of germination. Although the genes that partitioned into cluster VI were already downregulated during the early phase of germination, cluster V contains genes that were mainly downregulated during the late germination phase.

To identify any associations between the expression profiles and different biological processes and functions during *A. quisqualis* germination, the 1,536 differentially expressed genes were assigned with GO terms, based on sequence similarities to proteins in the SwissProt database using Blast2GO software (20). In total, 589 of the 1,536 sequences were assigned with one or more GO terms. Additionally, we searched for specific GO terms that were over- or underrepresented within the different groups of transcripts (Table 2; Supplementary Table 4). Our results revealed that genes grouped in cluster II, which have maximum expression levels in the early germination phase, are enriched with GO terms related to gene expression and translation ($P < 0.05$, Fisher's exact test with FDR correction), whereas terms related to catalytic activity are underrepresented (Table 2). In contrast, during the later phase of *A. quisqualis* germination, protein catabolism becomes more active. Cluster III, containing genes with an expression peak during the late germination phase, is enriched with terms related to proteasome biogenesis, proteolysis, and protein catabolism ($P < 0.05$, Fisher exact test with FDR) (Table 2). Moreover, cluster IV, which contains genes upregulated during the transition from early to later germination phase, is enriched with terms related to the metabolism of small molecules resulting from the breakdown of bigger molecules such as proteins and sugars (Table 2). Finally, cluster I, which consists of transcripts that were upregulated during the whole germination process (early and late phase), is enriched with terms related to protein transport and localization, whereas clusters V and VI did not show any statistically significant enrichment for any GO term.

Identification of genes with a putative biocontrol function in *A. quisqualis*. A striking feature of the transcriptional reprogramming of *A. quisqualis* was the identification of several genes encoding putative mycoparasitism-associated proteins. Among them we identified genes related to proteolysis and toxin biosynthesis, as well as virulence-related factors (Table 3). As shown previously, genes related to proteolysis (proteasome biogenesis and proteases) were substantially upregulated during the germination of *A. quisqualis*. Among these, there were the homologs of extracellular proteolytic enzymes such as dipeptidyl-peptidase 5 (Amp_20306 and Amp_4387), the secreted tripeptidyl-peptidase SED3 (Amp_5331), aminopeptidase 2 (Amp_2014), and the M6 family metalloproteases (Amp_14323 and Amp_26588). Virulence-related factors were also detected among the upregulated genes (Table 3). Among them were homologs of hydrolytic enzymes such as lipase 2 (Amp_22580) and endoglucanase IV (Amp_4729) that potentially could be involved in host cell-wall breakdown and a homolog of the snodprot1 secreted protein (Amp_13090). Interestingly, although no production of any toxin has been reported for *A. quisqualis* (39), we were able to identify transcripts coding for proteins related to the biosynthesis of toxic metabolites (Table 3). Among these there was a homolog of the Trichodiene oxygenase (Amp_26603) and a transcript related to Sterigmatocystin biosynthesis (Amp_19903). Finally, two putative genes related to efflux transporters, a homolog of the multidrug transporters family (Amp_13372) and a homologue of the Major facilitator superfamily (Amp_20430), were identified among the highly induced transcripts.

Identification of stored transcripts in dormant conidia. An interesting feature of the *A. quisqualis* transcriptome was the identification of a large number of stored transcripts in resting conidia. Our data showed that more than 10% (>3,000) of the transcripts represented on the microarray were present at high levels in dormant conidia based on their normalized array intensities (Supplementary Table 5). Interestingly, although some of the stored transcripts seemed to decay soon after germination of *A. quisqualis* conidia, the majority maintained a constant expression level during the germination process. It is worth mentioning that, among these, we were able to identify transcripts related to hydrolytic enzymes, lectins, and vacuolar proteases (Table 4). Furthermore, a sequence with homology to mitogen-activated protein kinase (MAPK) was detected among the most highly

TABLE 1. Transcriptome assembly statistics

Variables	Combined host recognition and early parasitization phase	Late parasitization phase
Total number of high quality sequences	283,139	321,121
Average length of sequences	203	200
Number of contigs	4,079	29,714
Number of singletons	59,825	40,100
Average length of contigs	258	252
Contig maximum length	1,376	1,034
Number of putative unique transcripts	63,904	69,814
Total number of bp covered by contigs	10,537,775	7,498,820

expressed transcripts in dormant conidia and maintained a constant expression level during the onset of germination.

Validation of microarray analysis using qRT-PCR. Eight transcripts were arbitrarily chosen and the expression levels were validated using qRT-PCR analysis. The magnitude of change determined by the qRT-PCR was in accordance with the microarray data for five of the eight genes considered (Supplementary Table 6). In addition, the expression of six more genes potentially

TABLE 2. Percentage of gene ontology (GO)-annotated transcripts within the different expression clusters in each of the three main GO categories

Category, GO term[a]	Process	Clusters[b] I	II	III	IV	V	VI	Total[c]
Biological_process	…	83.8	83.3	83.0	96.6	78.4	81.7	79.1
GO:0008152	Metabolic process	63.9	71.2	74.1	86.2	66.7	63.5	62.8
GO:0009987	Cellular process	61.3	71.2	50.4	65.5	23.5	60.6	53.6
GO:0051179	Localization	17.3	12.1	9.6	10.3	13.7	19.2	14.3
GO:0065007	Biological regulation	8.4	4.5	5.9	6.9	7.8	17.3	8.1
GO:0050896	Response to stimulus	6.3	1.5	2.2	0.0	3.9	1.0	4.4
GO:0044237	Cellular metabolic process	41.9	60.6	43.0	55.2	17.6	44.2	40.8
GO:0044238	Primary metabolic process	39.8	57.6	40.7	20.7	31.4	40.4	38.1
GO:0043170	Macromolecule metabolic process	31.9	**53.0**	27.4	6.9	19.6	26.0	27.9
GO:0044281	Small molecule metabolic process	12.6	10.6	23.7	**51.7**	9.8	18.3	14.8
GO:0051641	Cellular localization	**6.8**	1.5	1.5	0.0	0.0	1.0	2.2
GO:0006807	Nitrogen compound metabolic process	18.3	18.2	24.4	41.4	11.8	26.0	20.5
GO:0019538	Protein metabolic process	25.1	**36.4**	19.3	3.4	13.7	11.5	17.8
GO:0044260	Cellular macromolecule metabolic process	26.7	45.5	15.6	0.0	15.7	23.1	22.5
GO:0010467	Gene expression	13.6	**33.3**	8.1	0.0	5.9	3.8	10.8
GO:0044249	Cellular biosynthetic process	14.7	**27.3**	5.2	0.0	5.9	4.8	9.4
GO:0009059	Macromolecule biosynthetic process	14.7	**27.3**	5.2	0.0	5.9	3.8	9.3
GO:0044267	Cellular protein metabolic process	19.4	**31.8**	8.1	0.0	9.8	9.6	13.8
GO:0006412	Translation	11.5	**27.3**	5.2	0.0	5.9	2.9	8.2
GO:0015031	Protein transport	**7.9**	3.0	1.5	3.4	0.0	0.0	2.9
GO:0090304	Nucleic acid metabolic process	9.4	13.6	8.1	0.0	7.8	16.3	11.0
GO:0016070	RNA metabolic process	5.2	12.1	6.7	0.0	5.9	13.5	8.0
GO:0046483	Heterocycle metabolic process	5.2	1.5	8.9	6.9	3.9	5.8	5.4
GO:0006508	Proteolysis	4.2	4.5	**11.9**	3.4	3.9	2.9	3.6
GO:0009117	Nucleotide metabolic process	4.7	4.5	11.1	6.9	3.9	2.9	5.1
GO:0009308	Amine metabolic process	3.7	0.0	4.4	**34.5**	2.0	3.8	4.3
GO:0005975	Carbohydrate metabolic process	3.1	6.1	5.9	13.8	7.8	7.7	5.6
GO:0006139	Nucleobase-containing compound metabolic process	14.1	18.2	19.3	6.9	9.8	20.2	16.2
GO:0006464	Cellular protein modification process	3.1	3.0	2.2	0.0	3.9	6.7	4.6
GO:0006457	Protein folding	**5.2**	3.0	0.7	0.0	0.0	0.0	1.5
Molecular_function	…	87.4	86.4	95.6	96.6	94.1	90.4	87.9
GO:0005488	Binding	50.3	40.9	54.1	51.7	51.0	55.8	52.4
GO:0003824	Catalytic activity	56.5	*40.9*	74.1	89.7	72.5	55.8	59.3
GO:0005215	Transporter activity	9.4	4.5	6.7	3.4	11.8	9.6	8.1
GO:0005198	Structural molecule activity	5.8	**25.8**	1.5	0.0	2.0	0.0	5.4
GO:0016787	Hydrolase activity	23.6	16.7	31.9	17.2	17.6	12.5	21.1
GO:0036094	Small molecule binding	19.4	10.6	22.2	31.0	21.6	22.1	21.2
GO:0003676	Nucleic acid binding	16.2	24.2	10.4	3.4	11.8	19.2	14.2
GO:0016491	Oxidoreductase activity	13.6	4.5	18.5	**41.4**	31.4	11.5	15.0
GO:0016740	Transferase activity	11.5	16.7	11.9	17.2	15.7	18.3	15.0
GO:0043167	Ion binding	*7.9*	10.6	19.3	20.7	17.6	21.2	16.5
GO:0005515	Protein binding	6.3	3.0	2.2	0.0	0.0	2.9	3.1
GO:0048037	Cofactor binding	1.6	1.5	8.9	17.2	13.7	6.7	6.3
GO:0016829	Lyase activity	2.6	0.0	4.4	3.4	2.0	5.8	3.1
GO:0016874	Ligase activity	2.6	0.0	4.4	6.9	2.0	6.7	4.1
GO:0008233	Peptidase activity	5.2	6.1	**10.4**	3.4	3.9	1.9	3.7
GO:0030528	Transcription regulator activity	0.5	1.5	2.2	0.0	3.9	6.7	3.0
Cellular_component	…	53.4	60.6	37.0	20.7	35.3	46.2	44.8
GO:0016020	Membrane	18.3	15.2	10.4	6.9	21.6	20.2	17.4
GO:0005623	Cell	45.5	50.0	32.6	13.8	15.7	29.8	33.2
GO:0032991	Macromolecular complex	**23.6**	**37.9**	14.8	0.0	5.9	2.9	12.9
GO:0043226	Organelle	26.7	36.4	19.3	3.4	9.8	20.2	20.5
GO:0043234	Protein complex	**13.6**	7.6	13.3	0.0	2.0	2.9	6.3
GO:0043227	Membrane-bounded organelle	17.8	13.6	17.8	3.4	7.8	19.2	14.1
GO:0005622	Intracellular	44.5	50.0	31.9	10.3	15.7	29.8	32.3
GO:0043229	Intracellular organelle	26.7	36.4	19.3	3.4	9.8	20.2	20.5
GO:0005737	Cytoplasm	18.3	**31.8**	8.1	3.4	3.9	6.7	12.1
GO:0030529	Rribonucleoprotein complex	9.9	**31.8**	1.5	0.0	3.9	0.0	6.5
GO:0005739	Mitochondrion	6.8	6.1	4.4	3.4	0.0	1.9	3.5
GO:0005840	Ribosome	6.8	**25.8**	0.7	0.0	2.0	0.0	5.4
GO:0043232	Intracellular non-membrane-bounded organelle	10.5	**25.8**	2.2	0.0	3.9	2.9	8.1
GO:0005634	Nucleus	7.9	7.6	11.1	0.0	5.9	12.5	7.6
GO:0000502	Proteasome complex	**4.7**	0.0	**7.4**	0.0	0.0	0.0	1.0

[a] Note that due to the redundant nature of the Gene Ontology structure individual sequences can have multiple GO terms assignments.

[b] All numbers represent the percentage of the number of sequences assigned in each GO term/total GO annotated sequences within each cluster. Statistically significant enriched GO terms within the six expression clusters are marked in bold for overrepresented GO terms and marked as italics for underrepresented GO terms. Enriched GO terms were estimated with Blast2GO suite using false discovery rate correction and a cutoff of 0.05.

[c] Total spotted.

related to mycoparasitism was also studied using real-time PCR, confirming the microarray results. However, because these data are the subject of a separate publication (D. Angeli, A. Colombini, S. Siozios, and I. Pertot, *unpublished data*), they are not presented here.

DISCUSSION

Over the last few years, the use of antagonistic and hyperparasitic fungi as biocontrol agents for agricultural applications has received considerable interest. However, an in-depth understanding of the biology and molecular aspects of their mycoparasitic action is necessary to improve their biocontrol capacity. Different experimental approaches have been adopted to study molecular events underlying various mycoparasitic interactions (46,56,58,64,71,82). A common problem with these approaches arises from the multitrophic nature of mycoparasitic interactions, which complicates the development of EST libraries. By definition, a mycoparasite is a fungus that parasitizes another fungus. Hence, safe sorting of transcripts belonging to different fungi is difficult. In the case of the mycoparasitic relationship of *A. quisqualis* with powdery mildews, the obligate biotrophic nature of the host further complicates experimental approaches by not allowing the use of pure cultures. This problem is increased by the absence of genomic information for the microorganisms involved. Traditionally, suppression subtractive hybridization (SSH) has been the preferred method for studying mycoparasitic interactions at transcription level, especially for nonmodel species (15,56,58). Although SSH-based approaches seem to overcome the problems of multi-interaction systems to some extent, they do not allow for large-scale transcriptional analysis and are also laborious.

This study adopted a high-throughput transcriptome-wide sequencing approach, combined with microarray technology, in order to subtract and create a comprehensive transcript catalogue for *A. quisqualis* during mycoparasitism. The 31,222 sequences of this catalogue were then used to develop an expression microarray and study the transcriptional changes of the mycoparasitic response in *A. quisqualis* under simulated conditions. It is worth noting that nearly 50% of these sequences showed no significant similarity to known protein sequences. This finding reflects the scarce genomic resources for *A. quisqualis* and, by extension, our limited knowledge of mycoparasitic processes (46,56).

In response to the powdery mildew host, a total of 1,536 *A. quisqualis* putative genes showed significant changes in transcription during the germination process. Interestingly, most of these changes were observed during the early phase of *A. quisqualis* germination, between 3 and 12 h poststimulation. During this period, the first morphological changes were observed in *A. quisqualis* conidia. Our results suggest that the molecular machinery was already activated, resulting in extensive transcriptional reprogramming in *A. quisqualis* soon after host recognition. Rapid and extensive induction of gene expression during the conidial "activation phase" has also been reported for other

TABLE 3. Log2 fold change of *Ampelomyces quisqualis* transcripts potentially associated with the mycoparasitism process

ID	Log2 fold change[a]			Annotation	E-value	Cluster
	D–E	D–L	E–L			
Proteolysis[b]						
Amp_20306	0,257	1,398	1,141	Probable dipeptidyl-peptidase 5	1,00E-31	III
Amp_5331	2,67	1,677	-0,993	Probable tripeptidyl-peptidase SED3	6,00E-11	II
Amp_4387	1,439	0,979	-0,46	Probable dipeptidyl-peptidase 5	6,00E-17	II
Amp_18911	0,753	1,222	0,468	Probable dipeptidyl-peptidase 3	2,00E-53	III
Amp_14323	0,035	1,215	1,18	Putative uncharacterized (m6 family metalloprotease domain-containing)	9,00E-20	III
Amp_26588	-0,042	1	1,042	Putative uncharacterized (m6 family metalloprotease domain-containing)	2,00E-29	III
Virulence[c]						
Amp_13090	2,219	2,484	0,265	Protein SnodProt1	3,00E-38	I
Amp_22580	1,566	1,459	-0,107	Lipase 2	3,00E-07	I
Amp_4729	0,704	4,32	3,616	Endoglucanase IV	6,00E-21	III
Amp_3533	0,529	1,737	1,208	Xylanolytic transcriptional activator xlnR	6,00E-21	III
Amp_22954	0,782	1,68	0,898	Xylanolytic transcriptional activator xlnR	5,00E-28	III
Amp_10033	0,757	1,362	0,605	Rho GDP-dissociation inhibitor	3,00E-14	III
Amp_7579	1,667	2,039	0,372	Adenylosuccinate lyase	1,00E-16	I
Amp_19903	1,324	1,415	0,091	Probable sterigmatocystin biosynthesis P450 monooxygenase	4,00E-12	I
Amp_26603	1,655	3,127	1,472	Trichodiene oxygenase	2,00E-09	III
Amp_20430	1,493	1,142	-0,351	Major facilitator superfamily domain-containing protein	7,00E-51	II
Amp_13372	1,227	2,249	1,022	Multidrug transporter	7,00E-06	III

[a] Comparisons: D = dormant conidia, E = early germination phase, and L = late germination phase.
[b] Proteolysis related.
[c] Virulence and production of toxic metabolites related.

TABLE 4. Stored transcripts in *Ampelomyces quisqualis* conidia potentially associated with mycoparasitism

ID	Annotation	E-value	Rank[a]
Amp_10581	Similar to glycosyl hydrolase	4,0E-66	2
Amp_7367	Mitogen-activated protein kinase	1,0E-22	79
Amp_16248	Polyubiquitin	3,0E-40	80
Amp_3049	Probable glucan endo-1,3-β-glucosidase	3,0E-22	226
Amp_22128	Cutinase	7,0E-23	258
Amp_12808	Probable glucan endo-1,3-β-glucosidase	4,0E-06	260
Amp_2024	Vacuolar protease	2,0E-69	448
Amp_12364	Probable glucan endo-1,3-β-glucosidase	8,0E-30	584
Amp_29505	26S protease regulatory subunit 4	4,0E-33	760
Amp_13797	Lectin-C	3,0E-09	881
Amp_15620	Lectin	3,0E-10	1,112
Amp_19713	Vacuolar protease	2,0E-69	1,123

[a] Transcripts were ranked based on their normalized array intensities.

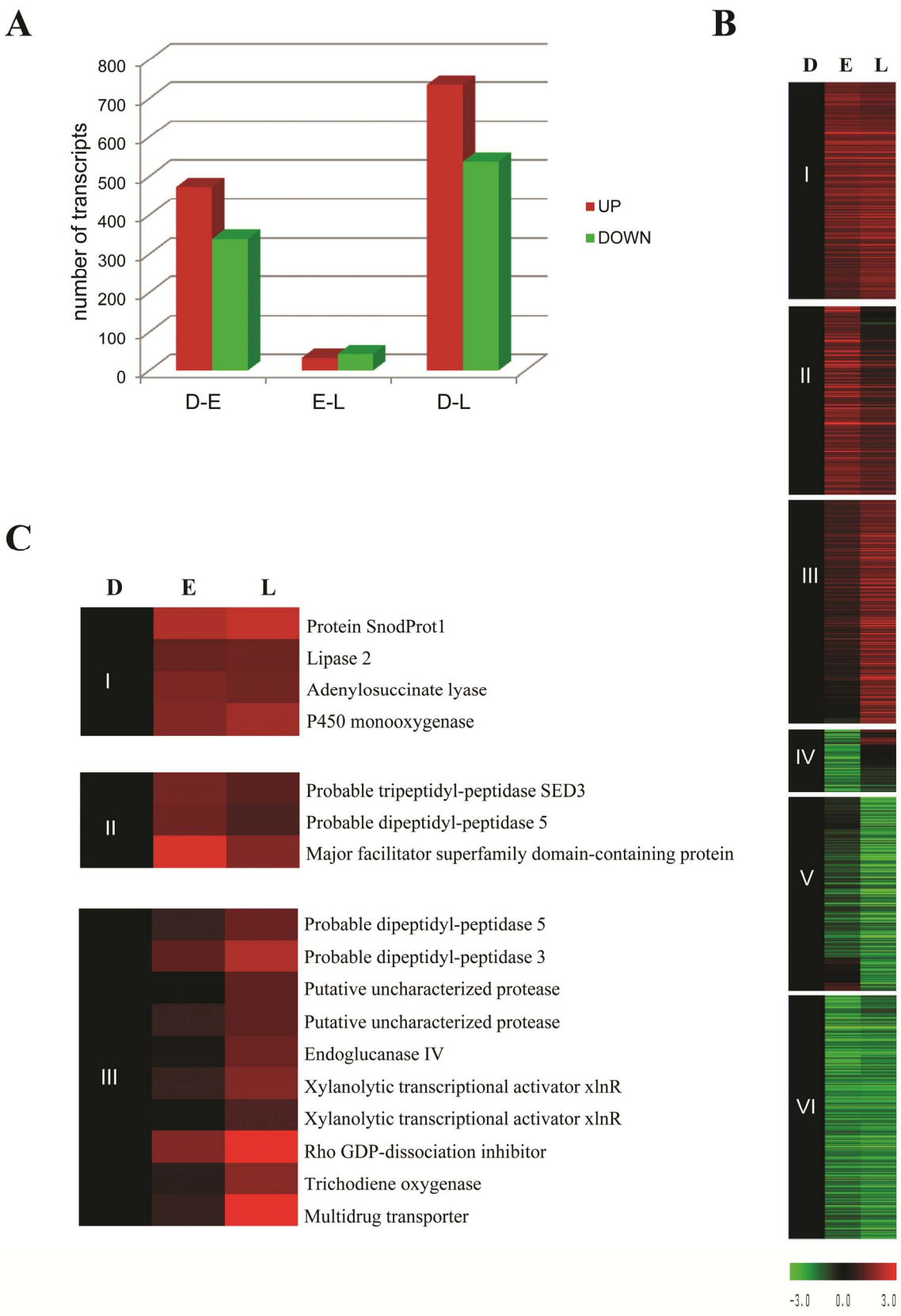

Fig. 1. Summary of microarray results. **A,** General transcription response of *Ampelomyces quisqualis* during host-induced germination. Each column represents the number of genes (sequences) whose expression changed at least twofold (UP or DOWN), compared with a previous time point. Bars D-E = comparison of early germination phase to dormancy, bars E-L = comparison of late germination phase to early germination phase, and bars D-L = comparison of late germination phase to dormancy. **B,** Heat map representation of six expression clusters identified by TIGR MultiExperiment Viewer (MeV). Upregulated sequences are shown in red and downregulated sequences in green. Each column corresponds to a different germination phase as labeled above: dormant conidia (D), early germination phase (E), and late germination phase (L). **C,** Expression profiles of differentially regulated transcripts of interest potentially involved in the *A. quisqualis*–*Podosphaera xanthii* interaction.

filamentous fungus and yeast species such as *Aspergillus fumigatus*, *Fusarium graminearum*, *Trichophyton rubrum*, and *Saccharomyces cerevisiae,* supporting our results (37,44,47,72). Functional classification of the differentially expressed genes also revealed that the early germination phase is enriched with transcripts involved in gene expression ribosome biogenesis and translation. There is convincing evidence that rapid protein synthesis is important for conidial germination in fungi (22,38,44, 47,61,72). These results suggest that rapid activation of the transcription and translation machinery is crucial for conidial transition from a dormant state to vegetative growth in *A. quisqualis*.

In contrast, during the later germination phase, we detected the largest number of genes related to protein catabolism. During this stage, polarized growth is established and germ tube elongation is evident. During this period, genes related to proteolysis and proteosomal degradation are among those most highly upregulated, suggesting that protein catabolism may become more active during the polar growth stage of the mycoparasite. This finding marks a large protein turnover during *A. quisqualis* mycelial development. Furthermore, during the germination period, genes associated with protein transport and localization are among the upregulated genes. Among them there are transcripts related to the Snf7 family of proteins, which are involved in protein sorting and transport to lysosomal compartments (63). Additionally, genes coding for secretion-related proteins are shown to be upregulated during this period. The above results suggest that both proteosomal and vacuolar proteolysis and the secretion machinery are activated, in order to facilitate the germination process but also the mycoparasitic response of *A. quisqualis* against powdery mildew.

Interestingly, during the transcriptional reprogramming of *A. quisqualis*, several upregulated genes were predicted to encode for potential mycoparasitism-related proteins. The role of secreted proteolytic enzymes in parasitism has been well documented in different mycoparasites and fungal pathogens (29,55,58,60). A recent survey on different *A. quisqualis* strains displaying different parasitic activities revealed a positive correlation between the production of proteolytic enzymes and the mycoparasitic activity of the strains (4). In line with the above results, our analysis revealed the upregulation of different proteases during *A. quisqualis* germination. Among them, we detected homologs of secreted proteases such as dipeptidyl-peptidase 5 and the tripeptidyl-peptidase SED3 and two putative genes with homology to the M6 family of metalloprotease domain-containing proteins. In order for *A. quisqualis* to get access to the host nutrient environment, digestion of the host cell wall constituents and the plasma membrane proteins is required. Secreted protease could facilitate this process and allow penetration of the host mycelium.

Notably, only one sequence related to cell-wall-degrading enzymes was detected among the upregulated genes and displayed homology with the endo-glucanase IV group of hydrolases. This group of hydrolytic enzymes is capable of hydrolyzing the β-1,4 glycosidic bonds in glucans, which are main constituents of the fungal cell wall (45). The importance and role of hydrolytic enzymes in mycoparasitism have already been suggested through direct or indirect evidence in different mycoparasitic systems (7,35,54,58). As regards the previous finding, two transcripts related to the xylanolitic transcriptional activator XlnR were also identified among the upregulated sequences during *A. quisqualis* germination. The transcriptional activator XlnR has been shown to be involved in the regulation of both xylanase- and endoglucanase-encoding genes in *Aspergillus niger* (84). Although none of the putative xylanase-encoding genes were found in our set of upregulated genes, we can speculate that the expression of the endo-glucanase in *A. quisqualis* is also regulated by XlnR in a similar manner to *Aspergillus niger*.

Several upregulated genes were predicted to encode pathogenicity and virulence-related factors. Among them, we identified a homologue of the lipolytic enzyme lipase 2. Lipases are enzymes that hydrolyze ester bonds of triacylglycerols, releasing fatty acids and glycerol, and have been implicated in pathogenic processes in both microbes and fungi (28,85). Secreted lipases are presumed to be involved in degradation of the host cell membrane, facilitating host penetration and establishment of parasitism, with the synergistic function of cell-wall-degrading enzymes (76). Another upregulated gene was identified as a homolog of the snodprot1 gene family, which encode for a group of small secreted proteins related to the cerato-platanin family (17,74). The Snodprot family is widespread among fungi, where it is believed to be involved in fungus–host interactions (17). Members of this protein family have been associated with phytotoxicity in different plant pathogens (36,62) whereas, in mycoparasitic species such as *Trichoderma atroviride* and *T. virens*, they have been identified as major elicitors of the plant defense response (25,70). The actual role of the snodprot1 homolog in the mycoparasitic action of *A. quisqualis* remains to be determined.

One of the strategies adopted by certain mycoparasitic fungi to antagonize or kill and utilize other fungi as a nutrient source is the production of several toxic metabolites (46,49,58). Characteristic examples are the role of trichothecens in the biocontrol potential of the *Trichoderma* spp. (49), as well as the role of macrosphelide A in the mycoparasitic interactions of *Coniothyrium minitans* with *Sclerotinia sclerotiorum* (52). Notably, no production of any toxins has been reported thus far for *A. quisqualis* (39). However, the production of several bioactive compounds has been reported from an endophytic *Ampelomyces* sp.-related isolate (1). Interestingly, we identified homologs corresponding to proteins involved in toxin biosynthesis among the upregulated genes. Among them, we identified a homologue of the trichodiene oxygenase, which has a key role in the trichothecene biosynthesis pathway (14), and a homologue of the sterigmatocystin biosynthesis P450 monooxygenase. The question of whether *A. quisqualis* is able to synthesize and utilize toxic metabolites during interaction with the powdery mildew host is intriguing and needs further investigation. Finally, two of the upregulated sequences matched with efflux transporter related proteins, such as multidrug transporters and the major facilitator superfamily. The bifunctional nature of these efflux transporters as both detoxification and drug resistance systems but also as exporters of secondary metabolites and toxins (19,24) suggests a putative role in the mycoparasitism process.

The presence of a large transcript pool in dormant conidia has already been shown in several studies and in different fungal species (13,44,47,72). The exact role of this abundant transcript pool in dormant spores remains largely unknown. However, it has been suggested that these transcripts could be primed for rapid translation, in response to appropriate stimulus such as the availability of nutrients or the presence of the host in the case of fungal parasites (61). Surprisingly, we found that around 3,000 transcripts were present at high levels in *A. quisqualis* dormant conidia. Most of this stored mRNA (around 70%) maintained constant expression levels throughout germination.

Among the most highly abundant transcripts in *A. quisqualis* conidia, there were sequences predicted to encode proteins related to cell-wall-degrading enzymes, including homologs of the glycosyl hydrolase family. Glycosyl hydrolases are a group of enzymes involved in the hydrolysis of chitin and glucan polymers, which are major structural elements in fungal cell walls (12,45). Although glycosyl hydrolases are known to have an important role in fungal development, including conidial germination or mobilization of energy reserves during germination, their direct involvement in mycoparasitism is also well documented (16,23,26,30). Enzymes such as glycosyl hydrolases are used by mycoparasites to degrade the host cell wall and get access to nutrients. Interestingly, a recent comparative genomic study between the mycoparasitic species of the *Trichoderma* genus re-

vealed a significant expansion of genes belonging to the glycosyl hydrolase family (42). This finding further supports the putative role of glycosyl hydrolases in mycoparasitism.

A homolog of the MAPK family was also detected among the most abundant transcripts in dormant conidia. This homolog shows significant similarity to the pathogenicity related MAPK 1 (Pmk1) of *Magnaporthe grisea* and the Tmk1 of *T. atroviride*. In fungi, MAPK signaling pathways are involved in the transduction of a wide variety of extracellular signals and play an important role in the regulation of different developmental processes, including those related to pathogenicity (88). Moreover, there is convincing evidence that MAPKs are directly involved in the establishment of parasitic interactions of different mycoparasites. Notably, a Pmk1 homolog from *T. virens* (Tvk1/TmkA) has been shown to be negatively involved in the regulation of different mycoparasitism-related genes, because the *tvk*1 null mutants showed increased levels of several lytic enzymes (53). As a result, the mutant strain presented increased biocontrol activity compared with the wild-type strain (53). Interestingly, and partially in contrast with the previous study, loss-of-function mutants of a *tvk*1 ortholog (*tmk*A) from a different *T. virens* strain showed decreased parasitic activity in direct conformation with the host (57). Similarly, although deletion mutants of the *tmk*1 gene from *T. atroviride* presented reduced ability to directly parasitize different hosts, they displayed increased biocontrol activity as a result of increased production of antifungal metabolites (65). Finally, two MAPK homologues from *C. minitans* (*CmBCK*1 and *CmSlt*2) were found to be positively involved in the parasitic activity of this hyperparasite, because null mutants showed a considerable reduction in parasitism (87). Rapid sensing and response to the presence of the powdery mildew host is important for the establishment of the mycoparasitic interactions by *A. quisqualis*. The presence of prestored mRNA coding for MAPKs could facilitate rapid translation and transduction of environmental signals.

Notably, two transcripts were predicted to encode for lectin-related proteins. Lectins are a well-known group of carbohydrate-binding proteins widely distributed in animals, plants, and microorganisms (43). Lectins have been found to be involved in different biological processes, including defense against predators and pathogens (10,21). Interestingly, lectins have also been implicated in molecular recognition and in symbiotic and parasitic interactions between different organisms (32,73). Studies suggest that lectins facilitate the symbiotic relationship between algae and fungi in lichens (50) and the *Rhizobium*–legume symbiosis (34,86). Moreover, in the case of the nematophagous fungi *Arthrobotrys oligospora,* molecular recognition, attachment, and subsequent hyphal penetration in the nematode prey is lectin mediated (59). *Ampelomyces quisqualis*-related lectins could potentially be involved in the mycoparasitic process in a similar manner, by recognizing the powdery mildew host and facilitating penetration.

In summary, this study represents the first large-scale genetic dataset for the mycoparasitic species of *A. quisqualis*. Our results provide us with a first glimpse of the molecular processes involved in the germination of *A. quisqualis* after recognition of the powdery mildew host. Furthermore, the study provides new insight into the mycoparasitic nature of *A. quisqualis* and reveals shared strategies deployed by different mycoparasites. Analysis of gene expression profiling revealed that *A. quisqualis* undergoes extensive transcriptional reprogramming, with 1,536 putative genes differentially regulated during germination. During this transcriptional activation, several mycoparasitism-related genes were identified. Although the actual function of these genes in *A. quisqualis* remains unknown, our data could be used to develop targets for further functional analysis, especially related to the biocontrol capacity of this mycoparasite. Future studies, including the sequencing of the *A. quisqualis* genome, could aid our understanding of the biology and evolution of the mycoparasitic lifestyle.

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