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Transcriptional landscape of trans-kingdom communication between *Candida albicans* and *Streptococcus gordonii*

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SUMMARY

Recent studies have shown that the transcriptional landscape of the pleiomorphic fungus *Candida albicans* is highly dependent upon growth conditions. Here using a dual RNA-seq approach we identified 299 *C. albicans* and 72 *Streptococcus gordonii* genes that were either up- or down-regulated specifically as a result of co-culturing these human oral cavity microorganisms. Seventy five *C. albicans* genes involved in responses to chemical stimuli, regulation, homeostasis, protein modification and cell cycle were statistically ($P \leq 0.05$) up-regulated, while 36 genes mainly involved in transport and translation were down-regulated. Up-regulation of filamentation-associated *TEC1* and *FGR42* genes, and of *ALS1* adhesin gene, concurred with previous evidence that the *C. albicans* yeast to hypha transition is promoted by *S. gordonii*. Increased expression of genes required for arginine biosynthesis in *C. albicans* was potentially indicative of a novel oxidative stress response. The transcriptional response of *S. gordonii* to *C. albicans* was less dramatic, with only eight *S. gordonii* genes significantly ($P \leq 0.05$) up-regulated ≥ twofold (*glpK, rplO, celB, rplN, rplB, rpsE, ciaR,* and *gat*). The expression patterns suggest that signals from *S. gordonii* cause a positive filamentation response in *C. albicans*, while *S. gordonii* appears to be transcriptionally less influenced by *C. albicans*.

Keywords

Cell wall proteins; adhesin; bacteria-fungi interactions; GPI anchor; RNASeq

INTRODUCTION

Candida albicans is a commensal fungus and opportunistic pathogen found in the human gut, oral cavity and genital tract. It is present in 20-60% humans, depending upon the population studied (Martins *et al.*, 1998). *C. albicans* can progress from commensal colonization to local invasion, according to subject susceptibility, and then to invasive candidiasis which is associated with high mortality rates (Pfaller *et al.*, 2010; Eggimann *et*

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al., 2015). In the oral cavity, *C. albicans* tends to localize to mucosal surfaces and prostheses e.g. dentures (Zomorodian *et al*., 2011), but there is also evidence for association with dental caries (de Carvalho *et al*., 2006) and periodontal disease (Canabarro *et al*., 2013). *C. albicans* therefore often coexists with other microorganisms in polymicrobial biofilm communities. There is evidence that this influences the morphogenetic, pathogenic and antifungal susceptibility properties of *C. albicans*, and therefore such infections may be more difficult to control (Wright *et al*., 2013).

Biofilm formation in *C. albicans* is a multistage process (Douglas, 2003) and is dependent upon morphological transitions from yeast to pseudohyphal or hyphal forms. The formation of biofilms involves global changes in gene expression (Garcia-Sanchez *et al*., 2004) modulated by at least six transcription factors (Nobile *et al*., 2012). A set of eight genes has been found forming a core filamentation response (Martin *et al*., 2013). The genes include *ALS3*, *ECE1*, *HGT2*, *IHD1* and *RBT1*, all of which encode cell wall proteins (CWPs).

Recently, RNA sequencing has been utilized to provide transcriptome maps of *C. albicans* under a range of growth conditions, both *in vitro* and *in vivo* (Bruno *et al*., 2010; Tierney *et al*., 2012; Grumaz *et al*., 2013). Such studies have revealed that relatively well established pathways may be affected unexpectedly by the prevailing growth conditions. For example, the arginine biosynthesis genes (e.g. *ARG1*, *ARG3*, *ARG4*) are induced under conditions of mild oxidative stress (0.5 mM hydrogen peroxide, H_2O_2) such as those which might occur within phagocytes (Jiménez-Lopéz *et al*., 2013).

In mixed species biofilms there are clearly many opportunities for trans-kingdom signalling to occur (Nobbs & Jenkinson, 2015) and it is therefore important to understand the nature of this communication. Quorum sensing molecules and other metabolites from Gram-negative bacteria such as *P. aeruginosa* (Hogan *et al*., 2004) and *Burkholderia cenocepacia* (Boon *et al*., 2008) have been shown to block hypha formation in *C. albicans*. *Staphylococcus aureus* appears to inhibit filamentation under some conditions (Fox *et al*., 2013), while the oral bacterium *Streptococcus mutans* inhibits hypha formation by production of trans-2-decenoic acid (Vilchez *et al*., 2010) and competence stimulating peptide (Jarosz *et al*., 2009).

Streptococcus gordonii is found associated with most surfaces in the human oral cavity (Aas *et al*., 2005) and is one of several oral streptococcal species that have been shown to co-aggregate with *C. albicans* (Jenkinson *et al*., 1990). It is suggested that these interactions with bacteria are crucial for *C. albicans* incorporation into oral cavity biofilms (Jenkinson, 2011), and for the development of polymicrobial communities. More recent studies have shown that one response of *C. albicans* to the presence of *S. gordonii* involves promotion of hyphal morphogenesis (Dutton *et al*., 2014). This could be influenced by cell-cell contact and modulated by secreted metabolites i.e. signalling molecules (Bamford *et al*., 2009; Jack *et al*., 2015). The aim of the work described in this paper was to outline the transcriptional responses of *C. albicans* and *S. gordonii* in the early stages of their interaction, mimicking the natural biofilm communication processes that occur in the oral cavity. The expression profiles obtained reveal how early recognition responses modulate downstream events involved in dual species, trans-kingdom biofilm development.

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METHODS

Growth of microbial cells

C. albicans wild-type strain SC5314 was grown aerobically for 16 h in YPD medium (2% yeast extract, 1% mycological peptone, 2% dextrose) at 37°C, with shaking at 220-rpm. Cells were then harvested by centrifugation (5000 × *g* for 5 min), washed twice in YPT medium (1 × Difco yeast nitrogen base, 20 mM phosphate buffer pH 7.1, 0.1% Bacto tryptone) by alternate centrifugation (5000 × *g* for 5 min) and suspension, and finally suspended at optical density 600 nm (OD_{600}) = 1.0 (approximately 1×10^7 cells ml^{-1}) in YPT medium. Aliquots (10 ml; 1×10^7 cells ml^{-1}) were transferred into conical flasks containing YPT medium (90 ml) supplemented with 0.4% glucose (YPT-Glc). The cultures were then incubated at 37°C for 2 h with shaking at 50-rpm to induce hypha formation (Dutton *et al.*, 2014). *S. gordonii* cells were grown anaerobically for 16 h in 10 ml BHY medium (per litre: 37g Brain Heart infusion broth, 5 g yeast extract) and then harvested by centrifugation (5000 × *g* for 7 min). The bacterial cells were washed twice with YPT (no glucose) and finally suspended at OD_{600} = 0.5 (2×10^8 cells ml^{-1}) in YPT-Glc medium.

Several combinations of *C. albicans*, *S. gordonii* and growth medium were designed to specifically identify changes in gene expression as a result of co-incubation (Table 1). For the dual-species cultures of *S. gordonii* and *C. albicans*, *S. gordonii* cell suspensions (50 ml; 2×10^8 cells ml^{-1}) were added at 2 h, while for the *C. albicans* monospecies culture, YPT-Glc medium alone (50 ml) was added. *S. gordonii* cell suspension (50 ml) was added to pre-warmed (37°C) YPT-Glc medium for 1 h for the *S. gordonii* monoculture. To prepare *C. albicans* spent medium, *C. albicans* cells were removed from the culture medium by centrifugation (5000 × *g*, 5 min), and the supernatant was vacuum filtered through a 0.45 μm nitrocellulose membrane. The filtered medium was transferred to a sterile glass bottle and warmed to 37°C before *S. gordonii* suspension (50 ml; 2×10^8 cells ml^{-1}) was added. All cultures were incubated at 37°C with shaking (50-rpm) for a further 1 h.

Cells were harvested by centrifugation (5000 × *g*, 10 min) in 50 ml-Falcon tubes and all but 5 ml supernatant was aspirated. The cell pellet was suspended in the remaining supernatant, transferred to sterile 15 ml-Falcon tubes and harvested by centrifugation (5000 × *g*, 5 min). The supernatant was aspirated until only 0.5 ml remained, and this was used to suspend the cell pellet. The cell suspension was frozen into small balls by dropping portions (200 μl) into liquid nitrogen. The balls were stored at −70°C prior to RNA extraction.

RNA extraction

Frozen microbial cell balls were thawed on ice and suspended in ice-cold RLT buffer (Qiagen Ltd., Manchester, UK) containing 2-mercaptoethanol and transferred to a sterile screw cap microfuge tube containing acid-washed Biospec glass beads (0.6 ml). The suspension was mixed with the glass beads and the fungal and bacterial cells were disrupted by alternating shaking (30 s) using a Fast-prep 25 bead beater (MP Biomedicals, Santa Ana, CA) and incubating 1 min on ice (repeated 3 times). The beads were allowed to settle and the supernatant was transferred to a sterile microfuge tube. The disrupted cells were centrifuged (13000 × *g*, 2 min) and the supernatant transferred to a sterile microfuge tube.

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An equal volume of 70% ethanol was added and the RNA was extracted and purified using an RNeasy Mini Kit (Qiagen) with the use of an on-column DNAse digestion (Qiagen). The quality of the RNA was checked by formaldehyde agarose-gel electrophoresis. The RNA concentration of each sample was measured spectrophotometrically (Nanodrop 1000, Thermo Scientific, Fisher Scientific UK Ltd, Loughborough, Leics., UK) and stored at −20°C.

Transcriptomic analysis

ERCC RNA Spike-In Control Mix (Ambion, Foster City, CA) was mixed with 2.5 μg RNA. Ribosomal RNA was depleted with a RiboZero Magnetic Gold Kit (Epicentre) and lllumina sequencing libraries were prepared using ScriptSeq v2 (Epicentre, Illumina Inc., Madison, WI) with 10 cycles of PCR amplification. The quality and quantity of each library was determined using a Bioanalyzer and the average (modal) insert size of samples was 400 bp with the spread ranging between 200 bp and 1,000 bp. An equimolar library pool was denatured, diluted to 6.5 pM and clustered on a cBot (Illumina) to create clonal clusters from single molecule DNA templates. One hundred base pair paired-end sequencing was undertaken using HiSeq2500 (Illumina) in high output mode with Truseq v3 reagents. The resulting FASTQ data were then filtered using the fastq-mcf command from the EA-Utils suite to remove adaptor sequences and low quality bases (Aronesty, 2011). The filtered data were then aligned against the reference ERCC transcripts using Bowtie v1.0.0 using the -X 600 flag. SAMtools v0.1.19 was utilized to convert the resulting SAM formatted-file to BAM (Li *et al.*, 2009). The number of reads mapping to each transcript was extracted using the SAMtools idxstats command and used to calculate RPKM values for each ERCC transcript. Log_2 values of observed RPKM were then plotted against log_2 expected RPKM values and inspected to establish lower limits of detection.

The reads which did not map to the ERCC transcripts were then aligned to a merged FASTA file containing both the Ca21_C_albicans_SC5314 genome (Version 21 from www.candidagenome.org) and the NC_009785 *S. gordonii* CH1 genome. The Tophat2 v2 2.0.8b program was used with the following parameters: -G -library-type fr-secondstrand -I 10000 -r 50, --mate-std-dev 100 -p 8. The -G parameter was followed by the combined gff file containing annotation for both organisms (Kim *et al.*, 2013). An additional analysis was carried out with the DESeq analysis tool to calculate differential gene expression (Anders & Huber, 2010). The output of Tophat2 was processed using the Bedtools multicov command (Dale *et al.*, 2011). This produces numbers of reads mapping to each annotated gff feature. The gene/read count files were then processed to separate the eukaryote and prokaryote gene features. Each was then analyzed separately using the DESeq package v1.12.1. Default parameters were utilized as outlined in the DESeq manual. DESeq uses a negative binomial model to account for the dispersion of the reads and the variation between replicates (n = 3), and uses a general linear model for comparisons (Love *et al*., 2014). P-values were calculated from DESeq, and Benjamini-Hochberg adjusted P-values ≤ 0.05 were deemed significant. All transcriptional data have been submitted to the GEO repository and assigned the GEO accession number GSE68477.

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Microscopy

Confocal scanning laser microscopy of *C. albicans* (stained with Calcofluor white) with or without *S. gordonii* (stained with fluorescein isothiocyanate, FITC) was performed as previously described (Dutton *et al.*, 2014). Transmission electron microscopy (TEM) was performed as follows: cell suspensions were centrifuged (5000 × *g*, 10 min) and to the pellets was added TEM fixative (4% paraformaldehye, 5% glutaraldehyde, 0. 1M sodium cacodylate buffer pH 7.2, 0.05% Tween 20). Tubes were shaken gently and incubated for 15 min at 22°C. The samples were centrifuged (3000 × *g*, 2 min), the supernatant carefully removed, and the pellet suspended in TEM fixative (2% paraformaldehye, 2.5% glutaraldehyde; 2 ml) for 16 h at 4°C. The pellets were then washed 3 times in 0.1 M sodium cacodylate buffer (pH 7.2), and set into low melting point 2% agarose. The agarose pellets were cut into ~3 mm sections and incubated for 1 h at 22°C in 1% osmium tetroxide solution. The samples were rinsed in sterile H_2O, dehydrated at room temperature using sequential incubations in ethanol (30%, 50%, 75%, 90% and 100%) then propylene oxide, and embedded in Spurr resin. The resin was cut by microtome (RMC Powertome PC) into sections of 80-100 nm using a diamond knife, and sections were collected on copper grids and imaged at 80 kV by TEM.

Scanning Probe Microscopy (SPM) was performed on *C. albicans-S. gordonii* cultures deposited onto glass cover slips. Cover slips were attached to metal pucks with double sided tape and mounted on a Multimode AFM fitted with a Nanoscope IIIa controller (Veeco, Santa Barbara, CA). Cells were imaged in contact mode using triangular silicon nitride (Si_3N_4) tips with a nominal spring constant of ~0.06 N m^{-1} (Veeco). Images were obtained at typical scan rates of 25 μm s^{-1} and processed using Nanoscope 8 software (Veeco).

RESULTS AND DISCUSSION

C. albicans and *S. gordonii* form close physical associations

Coaggregation of *C. albicans* and *S. gordonii* cells occurs rapidly after mixing of the two cell types (Jenkinson *et al*., 1990). In order to visualize these interactive events, *S. gordonii* cells, fluorescently labelled with FITC, were mixed with hyphae-forming *C. albicans* cells in YPT-Glc medium (see Methods) and incubated for 1 h at 37°C. The associations between the bacteria and fungi were then viewed by confocal scanning laser microscopy (CSLM). After 3 h growth in monoculture, ~50% *C. albicans* cells had formed hyphae of 20-50 μm in length (Fig. 1A). In the presence of *S. gordonii,* bacterial cells bound along the lengths of the hyphal filaments (Fig. 1B) and could also be seen to aggregate, forming microcolonies at discrete attachment sites (Fig. 1B). These aggregates must form mainly by recruitment of other streptococci since extensive bacterial cell division does not occur in YPT-Glc medium within the experimental time-frame of 1 h.

One characteristic of *C. albicans* hypha formation is that the hyphal filaments clump together. As expected therefore, transmission electron micrographs showed *C. albicans* hyphal filaments in close contact with each other (Fig. 2A), seemingly connected by networks of fibrillar material. *S. gordonii* cells were often seen intimately interacting with hyphal filaments, with the cell surfaces in direct contact along a length of about 100 nm

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(Fig. 2B). There were also streptococci present that were not interacting with hyphae (Fig. 2B). The closeness of the bacterium-fungus interaction was further shown by scanning probe microscopy. In scans of wet mounts, streptococcal and *C. albicans* cell surfaces are apparently coalesced (Fig. 3A), while under dried conditions there appears to be a structural difference on the hyphal cell surface at the point of contact with bacteria (Fig. 3B) which could suggest some form of fungal cell wall remodelling. It is these close associations between the two cell types that led us to hypothesize that recognition signals (contact or diffusible) could be relayed through cell wall sensors to modulate gene expression in response to the other microorganism.

Transcriptional landscape studies

Following suspension of *C. albicans* cells in YPT-Glc medium, and incubation for 2 h at 37°C, fungal cells were undergoing early-stage hyphal morphogenesis (see above). At this point the *C. albicans* cells were allowed to proceed for a further 1 h, either in the presence or absence of *S. gordonii*, or in the presence of spent *C. albicans* culture medium (to correct for metabolic effects), and RNA was then prepared. Transcriptomic data therefore represent the response of *C. albicans* to the presence of *S. gordonii* for 1 h, and they take into account also the transcriptional effects of nutritional shift-down and *C. albicans* culture medium on *S. gordonii* cells (see Table 1). Under these conditions, physical trans-kingdom interactions occur, and chemical signals would carry on being exchanged between the two microorganisms since both *C. albicans* and *S. gordonii* continue to metabolize within this medium (Bamford *et al*., 2009).

Illumina sequencing from the combined *C. albicans* and *S. gordonii* samples yielded 258,347,928 raw reads (Table S1). A total of 6,653 open reading frames (features) from all the samples were functionally annotated to the haploid assembly 21 of the *C. albicans* genome (van het Hoog *et al.*, 2007) using the *Candida* Genome Database (CGD) (www.candidagenome.org), while 2,051 open reading frames were matched to the *S. gordonii* database for annotation, visualization and integrated discovery (DAVID version 6.7 - http://david.abcc.ncifcrf.gov) (Huang *et al.*, 2007; Sherman *et al.*, 2007). The total numbers of candidal and streptococcal reads for all three sample replicates were calculated, and the % distribution of candidal and bacterial reads were calculated for the three combined replicates for each sample (Table S2).

When *C. albicans* and *S. gordonii* were co-cultured in YPT-Glc medium, the overall ratio of reads was 37.7% *C. albicans* to 62.7% *S. gordonii*. This showed there was a suitable distribution of *C. albicans* and *S. gordonii* reads with no undue bias towards one single organism. It should be noted that features can include any sequence belonging to the genome, including reads that are not translated into proteins. This explains why there were a small number of reads (0.09% and 0.03%) from the *S. gordonii* samples that matched to the *C. albicans* genome. These relate to regions of the genome with some homology that are similar in the two organisms e.g. tRNA or mitochondrial RNA.

One technical consideration was if there might be a bias towards long or short transcripts. To check this, the gene (orf) coordinates from the 6,653 orfs in the CGD were used to prepare a dataset of the lengths of every orf. The normalized expression reads (tag counts)

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for every gene were then plotted against gene length for the entire *C. albicans* genome. The plot was compared with a corresponding graph of normalized expression reads versus gene length for the up- and down-regulated *C. albicans* genes in the presence of *S. gordonii*. These data, presented in Fig.S1, show that there was no shift in length distribution between total *C. albicans* orfs and differentially regulated genes, and no obvious change in range of distribution. Therefore it was concluded that the expression data were not biased by gene length.

The Illumina sequencing data from co-incubated cultures of *C. albicans* and *S. gordonii* were compared to the data obtained from *C. albicans* grown with only the addition of growth medium (YPT-Glc) minus *S. gordonii*. This was to rule out the likelihood that any changes in gene expression were caused by the addition of the extra growth medium after 2 h rather than an effect caused by *S. gordonii* cells. Volcano plots of *P*-value vs. mean fold change in gene expression (Fig. 4) derived from analysis of the Illumina data (by the statistical DESeq package v1.12.1 with default parameters) showed that when *C. albicans* and *S. gordonii* were co-cultured in YPT-Glc medium the expression levels of a large number of *C. albicans* genes were significantly ($P \leq 0.05$) up- or down-regulated by at least a twofold change (Fig. 4A, Table 2). On the other hand, only one *S. gordonii* gene was significantly ($P \leq 0.05$) decreased in expression when the bacteria were incubated with *C. albicans*. *S. gordonii* gene expression significantly increased only slightly overall, with the majority of increases around twofold or less (Fig. 4B). Statistical analysis using DESeq v1.12.1 also showed the total number of genes with altered expression (either up- or down-regulated) was much higher in *C. albicans* (299 genes) compared with *S. gordonii* (72 genes).

Table 2 shows all genes identified as significantly ($P \leq 0.05$) up- or down-regulated by DESeq in *C. albicans* when hypha-forming cells were incubated with *S. gordonii* for 1 h. Eighteen out of 75 genes up-regulated ≥ twofold are annotated as being associated with stress responses (core, oxidative, acid, macrophage up-regulated), while 15 genes are annotated as being up-regulated in biofilm formation (Table 2). Only one up-regulated gene (*TSA1*) was associated with both stress and biofilm formation, suggesting that at least two response pathways were being activated. These results, together with the volcano plots, indicated that when *C. albicans* and *S. gordonii* were co-incubated there was a much larger overall effect on *C. albicans* compared to a relatively small effect on *S. gordonii*.

GO Biological Processes

Within the Gene Ontology (GO) category for Biological Processes, from 152 *C. albicans* up-regulated genes (Table 2), 75 were significant ($P \leq 0.05$), while 36 of the 147 down-regulated *C. albicans* genes were found to be significantly ($P \leq 0.05$) down-regulated. The genes with significant differential gene expression were assigned to 41 GO Slim categories (Fig. 5). The numbers of genes assigned to *biological process, other* and *chemical stimulus* (the most abundant) were all found to be significantly ($P \leq 0.05$) affected (Fig. 5). From the *C. albicans* down-regulated genes the terms *biological process* and *transport* were the most abundant (25%) and the terms *other* and *translation* were the next highest in abundance

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(13.9%). The genes assigned to *transport* were found to be significantly (*P* ≤0.05) affected (Fig. 5).

GO Cellular Components

Cellular Components describe locations, at the levels of subcellular and macromolecular complexes. For GO category Cellular Components, 75 up-regulated *C. albicans* genes were assigned to 27 Slim categories (Fig. 6). From the up-regulated *C. albicans* genes the term *cytoplasm* (48.6%) was the most abundant with the terms *cellular component* (37.8%) and *mitochondrion* (28.4%) showing the next highest abundance. Gene ontology terms associated with the *nucleus*, *mitochondrion*, and *cell wall* were all found to be significantly (*P* ≤0.05) enriched in the up-regulated gene sets (Fig. 6). For the down-regulated *C. albicans* genes, GO Slim terms assigned to *cytoplasm* and *cellular components* were found to be significantly (*P* ≤0.05) affected (Fig. 6).

GO Molecular Functions

Molecular Functions describe activities, such as catalytic or binding activities that occur at the molecular level. For the GO category Molecular Function, the 75 significantly up-regulated *C. albicans* genes (from a total of 152 up-regulated genes) (Fig. 7) and 36 significantly down-regulated *C. albicans* genes were assigned to 26 GO Slim categories. From the up-regulated *C. albicans* genes, the term *molecular function* (36.5%) was the most abundant, with the terms *oxidoreductase activity* (25.7%) and *transferase* activity (14.9%) showing the next highest abundance. The number of genes assigned to *oxidoreductase activity* and *hydrolase activity* were found to be significantly (*P* ≤0.05) affected (Fig. 7). From the *C. albicans* down-regulated genes the term *molecular function* (27.8%) was the most abundant, with *oxidoreductase activity* (16.0%) and *transporter activity* (16.7%) significantly (P≤0.05) affected.

Colonization and pathogenesis genes

From 211 genes associated with pathogenesis by CGD, only 10 genes were shown to be up- or down-regulated by ≥ twofold (Table 3). The genes *ALS1*, *CAT1* and *TEC1* are strongly associated with the transition from yeast to hyphae during filamentous growth as well as pathogenesis. The up-regulation of *TEC1* in this study tends to suggest that *S. gordonii* stimulation of *C. albicans* hyphal growth might occur through the Cyr1/cAMP signalling pathway (Sudbery, 2011).

For successful colonization and pathogenesis, once *C. albicans* blastospores adhere to host surfaces they rapidly begin to produce hyphal filaments. Hyphae physically penetrate host endothelial and epithelial cells. Aided by a large array of hyphal surface proteins they also create stronger attachments to the host surfaces. Overall there were 18 genes associated with filamentous growth whose expression was affected (six significantly) when *C. albicans* was challenged with *S. gordonii* (Table 3). *FRG42*, *ALS1*, *CAT1*, and *TEC1* each showed a mean > four-fold increase. *ALS1* and *TSA1* transcripts were of highest abundance (Table 3). Tec1 and Fgr42 proteins are both filamentous growth regulators (Sudbery, 2011), so are involved in the switch from yeast form to hyphal form, suggesting that the gene expression network associated with the yeast to hypha transition was stimulated by the presence of *S. gordonii* in

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the culture. This is supported by evidence for growth synergy of streptococci and *C. albicans* in biofilms (Bamford *et al.*, 2009) and morphological observations of more extensive hyphal filament formation with *S. gordonii* present (Dutton *et al.*, 2014).

Oxidative stress related genes

Up-regulation of *CAT1* (catalase) during the yeast-hypha transition might be consistent with reports that reactive oxygen species (ROS) levels are increased under hypha-forming conditions (Schroter *et al.*, 2000). During the transition of yeast to hyphal filament, the cell wall must go through a considerable conformational change, involving restructuring, which ultimately requires a greater energy input from the cell. The energy comes from oxidative phosphorylation in mitochondria, which generate ROS as a by-product. The ROS will attack and inhibit the functions of proteins, lipids and DNA if not decomposed by Cat1 and other anti-oxidant enzymes. So an increased energy output during hypha formation will generate a need for an increase in anti-oxidants.

HSP21, one of the up-regulated genes in Table 3, encodes a multifunctional heat shock protein, with a role in both stress adaptation and virulence in *C. albicans* (Mayer *et al.*, 2012). Hsp21 modulates thermal stress by fine tuning homeostasis of compatible solutes and activation of the Cek1 pathway, which has previously been shown to be influenced by *S. gordonii* (Bamford *et al*., 2009). Hsp21 also mediates adaptation to oxidative stress, while an *hsp21*Δ/Δ mutant forms shorter hyphae than the wild-type and is strongly attenuated in virulence *in vivo* (Mayer *et al.*, 2012).

Numerous *C. albicans* anti-oxidant genes in addition to *CAT1* responded to the presence of *S. gordonii* including *ORF19.3537* a putative sulfiredoxin, two oxidoreductases (*ORF19.2262* and *CIP1)*, cytochrome c peroxidase (*CCP1*), glutathione reductase (*GLR1*), glutathione-S-transferase (*GTT11*), glutathione peroxidase (*GPX2*), thioredoxin reductase (*TRR1*) and the thiol-specific antioxidant protein (*TSA1)*. *TSA1*, *TRR1*, *CAT1*, *GLR1* and *CPA2* transcripts were of high abundance (Table 2). However, not all known oxidative stress response genes were up-regulated e.g. *TRX1*, *SOD1*, *GRX2*, *GPX31-33* (da Silva Dantas *et al.*, 2015) suggesting a more specific type of response to *S. gordonii.* Many of the antioxidant genes are reported to be regulated by Cap1 (Wang *et al*., 2006) and their products are up-regulated upon H_2O_2 treatment of *C. albicans* (Kusch *et al*., 2007). Overall, protection against H_2O_2 potentially confers added resistance to macrophages and cells of the innate immune system that utilize oxidative stress to kill *C. albicans*.

ARG1, *ARG3*, *ARG4*, *ARG5,6*, *CPA1*, and *CPA2* are all involved with arginine biosynthesis are also greatly up-regulated in *C. albicans* with *S. gordonii* present. Conversely *CAR1*, an arginase, is strongly down-regulated suggesting that increased arginine production is important for the response of *C. albicans* to *S. gordonii*. Since the *ARG* genes are up-regulated in the presence of H_2O_2 (Jiménez-Lopéz *et al*., 2013) these effects could be in direct response to the production of H_2O_2 by *S. gordonii* (Liu *et al*., 2011). The importance of arginine biosynthesis is further strengthened by the up-regulation of *PUT1*, a putative proline oxidase that is expected to convert L-proline into Δ^1-pyrroline-5-carboxylate and glutamate-γ-semialdehyde that act as the main precursors for arginine production and

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polyamine production. In *Saccharomyces cerevisiae*, exposure to H_2O_2 and freeze-thaw stress also leads to an accumulation of arginine (Almeida *et al.*, 2007; Momose *et al*., 2010), with supplementary arginine conferring resistance to oxidative and thermal stress (Nishimura *et al*., 2010).

Covalently linked cell wall proteins (CWPs)

The *C. albicans* cell wall consists of an internal scaffold of (β-1,3)- and (β-1,6)-linked glucan and chitin, to which an outer protein coat is attached (Klis *et al.*, 2001; Ruiz-Herrera *et al.*, 2006). The protein coat is thought to contain about 20 different polypeptides attached to the cell wall by covalent bonds linking proteins to the inner glucan/chitin skeleton (Klis *et al.*, 2009). These cell wall proteins (CWPs) have been associated with many functions including host cell adhesion and invasion, biofilm formation, cell-cell and intergeneric aggregation, and enzymatic functions such as superoxide dismutases and yapsin-like aspartic proteases (Monod *et al.*, 1998; Martchenko *et al.*, 2004; Krysan *et al.*, 2005). Because of the diversity of their roles, it is not surprising that expression of CWP-encoding genes can vary enormously, not only with mode of growth, but also with environmental signals and input from distinct signalling pathways, triggered by changing environmental conditions (e.g. temperature, pH, *N*-acetyl-D-glucosamine etc.). This analysis investigated 36 experimentally-validated covalently-linked CWPs of *C. albicans* (Klis *et al.*, 2009) and how their gene expression profiles changed when *C. albicans* cells were co-incubated with *S. gordonii* (Table S3).

Thirteen out of 18 *C. albicans* CWP genes showed ≥ twofold up-regulation of gene expression, while five were ≥ twofold down-regulated (Table 4). Chi-squared tests showed that cell wall protein genes *PGA57*, *ALS1*, *PGA34*, *PHA36* (*IHD1*), *PGA61*, and *ORF19.4653* were all significantly up-regulated ($P \leq 0.05$). *PGA10* was up-regulated > four-fold, though this was not considered significant at $P \leq 0.05$. Pga10 (Rbt51) belongs to a sub-set of fungal proteins with an eight cysteine residues domain CFEM (Common in several Fungal Extracellular Membrane proteins). Pga10/Rbt51 along with other proteins Rbt5 and Wap1/Csa1, each contain CFEM domains, which play key roles during biofilm formation (Perez *et al.*, 2006).

The up-regulation of expression of *HYR1* is most likely to be associated with an increase in the extent or rate of hypha formation. Studies have shown *HYR1* is induced specifically in response to hyphal development when morphogenesis is stimulated by growth conditions such as serum, temperature elevation, pH and the addition of *N*-acetyl-D-glucosamine (Bailey *et al.*, 1996). The findings suggest that the just over twofold increase in expression of *HYR1* could be associated with yeast to hypha transition, as shown by Spiering *et al*. (2010), stimulated by the addition of *S. gordonii* to the culture.

GPI-modified proteins

Glycophosphatidylinositol (GPI)-modified proteins all share conserved features of an N-terminal signal sequence and C-terminus tethered to the cell wall or cell membrane by a preformed GPI anchor. *In silico* predictions suggest that there are 115 genes encoding GPI-modified proteins in *C. albicans* (Richard & Plaine, 2007). Several lists of *C. albicans* GPI-

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modified proteins (GpiPs) published in the literature (De Groot *et al.*, 2003; Garcera *et al.*, 2003; Eisenhaber *et al.*, 2004) have been amalgamated and refined (Richard & Plaine, 2007) to avoid duplications and allow for differences in the algorithms used to define GpiPs.

The functions of the majority of these GpiPs (66%) still remain unknown. The others can be assigned to functions related to cell wall biosynthesis or remodelling, and cell-cell adhesion and interactions. Since the compilation of this list of GpiPs, 45 knock-out mutants of computer predicted GpiPs have been screened for their roles in cell wall structure (Plaine *et al*., 2008). Deletion mutants that result in cell wall modifications and reduced caspofungin sensitivity included *DFG5*, *PHR1*, *PGA4* and *PGA62*.

In our data, from the 115 predicted GpiPs, only 16 had a change in expression > twofold in the presence of *S. gordonii* (Table S4). A small number of genes (five) were down-regulated while 11 genes showed up-regulation (Table S4), six of which were significant (P <0.05). These were *ALS1*, *ORF19.4653*, *PGA34*, *PGA36*, *PGA57* and *PGA61* (Table 4). The CGD provides information on *PGA34* (role in host infection) and *PGA36* (induced during hyphal development), while *ORF19.4653*, *PGA57* and *PGA61* are currently uncharacterized but clearly respond transcriptionally to *S. gordonii*.

Adhesins

C. albicans cells within the oral cavity must avoid being washed away by the continuous flow of saliva, therefore adhesion to a multitude of host surfaces including epithelial cells, teeth, and dentures is of paramount importance. *C. albicans* possesses numerous potential adhesins (Zordan and Cormack, 2012) and this part of the study investigated 23 proteins which have been linked to cell adhesion in the CGD.

When *C. albicans* was co-incubated with *S. gordonii*, 19 out of the 23 *C. albicans* cell wall protein genes associated with adhesion showed up-regulation of gene expression, while four were seen to be down-regulated (these were all less than twofold changes). Both *ALS1* and *TEC1* showed the highest mean fold change in up-regulated adhesion-associated genes with significant four-fold changes (P ≤0.05) (Table 4). *ALS1* expression is known to be associated with hypha formation and, on the emergence of the germ tube, Als1 is the first member of the Als family to be expressed. Interestingly, the expressed protein is localized at the neck of the growing hypha (Fu *et al*., 2002). Differential expression patterns of the *ALS* genes have shown that there is a major spike in *ALS1* expression when cells are inoculated into fresh medium, a procedure which also triggers hyphal growth, indicating that Als1 might have a regulatory role as well as an adhesin function. The up-regulation of *TEC1*, a TEA/ATTS transcription factor, is consistent with its reported role in regulating hypha formation and virulence (Schweizer *et al.*, 2000).

EAP1 (2.6-fold increase), *HIS4* (three-fold increase) and *HYR1* (2.1-fold increase) were also seen to be up-regulated. Eap1 is a glucan-cross-linked cell wall-localized protein that has been reported to be required for *C. albicans* to form robust biofilms on polystyrene surfaces (Nobbs *et al.*, 2010) and in central venous catheters (Li *et al.*, 2007) under shear flow *in vitro* and *in vivo.* Although expressed in both yeast and hyphal cells, it has been suggested that Eap1 protein expression is not directly associated with hypha formation (Li and

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Palecek, 2003). However, there are contradicting reports where transcriptional profiling studies of the yeast to hypha transition reveal a twofold increase in Eap1 at 6 h (Nantel *et al.*, 2002) similar to the approximately three-fold up-regulation seen here. Interestingly, studies of *S. cerevisiae s*trains expressing Eap1 have confirmed that Eap1 is able to bind *S. gordonii* in planktonic culture (Grubb *et al.*, 2009). This indicates that Eap1, along with a number of other adhesins such as Als3 and Hwp1, promotes trans-kingdom interactions with other microorganisms to aid successful colonization and the formation of polymicrobial communities (Nobbs *et al.*, 2010; Xu *et al*., 2014). Although *EAP1* expression was up-regulated, expression of the genes encoding other *C. albicans* adhesins *ALS3* and *HWP1* was not over the time-course of the experiment.

S. gordonii transcriptional response to *C. albicans*

A total of 72 *S. gordonii* genes were either up- or down-regulated specifically in response to co-culturing with *C. albicans* (Table 5). Eighteen *S. gordonii* genes were significantly up-regulated including ≥ twofold increases in *glpK*, *rplO, celB, rplN, rplB, rpsE, ciaR, and gat* (Table 5). Glycerol kinase (GlpK) allows glycerol to be utilized as a carbon source. Notably, a highly up-regulated gene in *C. albicans* biofilms was *RHR2* encoding the glycerol biosynthetic enzyme glycerol-3-phosphatase. Glycerol is five times more abundant in *C. albicans* biofilm cells (Desai *et al.*, 2013) so it seems possible that *S. gordonii* may respond to glycerol production or secretion by *C. albicans* hypha-forming cells by utilizing this as an alternate carbon and energy source.

CiaR is a response regulator of competence for DNA uptake (Mascher *et al.,* 2003) and biofilm formation (Blanchette-Cain *et al.,* 2013) in *Streptococcus pneumoniae.* We have shown recently that *S. gordonii* competence-development is involved in formation of dual species biofilms with *C. albicans* (Jack *et al*., 2015), and so CiaR may be a factor in this process. *cel* genes annotated as encoding components of cellobiose metabolism were up-regulated, perhaps indicating that *S. gordonii* is responding to the presence of *C. albicans* cell wall glucans. Up-regulation of *rplO, rplN, rplB,* and *rpsE,* all involved in translation, may be an artefact of ribosomal depletion. Only *glgP-2* (maltodextran phosphorylase) was significantly down-regulated (Table 5). Regions of the *S. gordonii* genome containing ORFs SGO_1105 to SGO_1108 showed coordinated down-regulation, and are all implicated in pyrimidine biosynthesis. Overall, these results suggest that *S. gordonii* was transcriptionally much less reactive to the presence of *C. albicans* for 1 h in mixed culture, while the effects of CiaR up-regulation in *S. gordonii* are a topic of future investigation.

Summary and conclusions

This work describes the responses of *C. albicans* and *S. gordonii* to each other at the transcriptional level. The experiments were undertaken under growth conditions that induce filamentation in *C. albicans.* The results presented suggest that *S. gordonii* has a range of significant effects on the biological processes occurring within *C. albicans* during the early phase of co-culture, with a large number of genes affected in *C. albicans* in comparison to a smaller number in *S. gordonii*. Genes involved in responses to chemical stimuli, regulation, homeostasis, protein modification and cell cycle were up-regulated, while genes involved in

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transport and translation were down-regulated. These patterns suggest that *C. albicans* was responding positively to signals produced by *S. gordonii*. Mitochondrial genes were up-regulated together with genes encoding cell wall proteins, suggesting triggering of metabolic functions.

On the other hand, down-regulation of triplet codon-amino acid adaptor and transporter activities suggest modulation of the rate of protein synthesis. Oxidoreductase activities were up- and down-regulated, while hydrolase activity genes were up-regulated, suggesting perhaps that new macromolecular substrates e.g. polysaccharide, peptidoglycan etc. were now available for metabolism by *C. albicans*. Overall the data are consistent with *C. albicans* not being growth-inhibited in the presence of *S. gordonii*, unlike the inhibitory effects of some other bacteria (e.g. *P. aeruginosa*) on growth and hyphal development (Hogan & Kolter, 2004; Fox *et al*., 2013). There was some evidence of up-regulation of genes that might be linked with growth-stimulatory effects, supporting previous observations from biofilm experiments (Bamford *et al.*, 2009; Dutton et al., 2014; Xu *et al.*, 2014).

The major changes in expression of morphogenesis-related genes in response to *S. gordonii* were upregulation of *TEC1*, *ALS1*, and *CAT1*. Notably, Tec1 is a hyphal-development activator (Nobile & Mitchell, 2005) that regulates expression of cell wall protein genes, but probably not *ALS1* (Nobile & Mitchell, 2006). *ALS1* also appeared in the up-regulated genes encoding covalently-linked CWPs and GPI-modified proteins. Clearly *TEC1*, *ALS1* and anti-oxidant genes seem to be major players in the response of *C. albicans* to *S. gordonii*. The Als1 protein is not thought to be a component of the hyphal cell wall, but appears at the initial site of hyphal filament growth from the mother cell (Coleman *et al.*, 2012). *ALS1* has been shown to be one of the genes that is first up-regulated following adhesion to a surface (Garcia-Sanchez *et al.*, 2004) that then leads onto biofilm formation. Further work is required to establish the factors affecting expression of *ALS1* and if it is involved in regulation as well as adhesion. Of the GPI-modified proteins, six genes (including *ALS1*) encoding these were significantly ($P \leq 0.05$) up-regulated in the presence of *S. gordonii*. The functions of the other five *PGA* (Protein with Glycosylphosphatidylinositol Anchor) genes are unknown. Further studies could help to identify the functions of the proteins encoded by these genes and they may reveal new factors for adhesion, biofilm formation and pathogenesis. In summary, these transcriptional data findings seem to correlate with the biological data indicating that *S. gordonii* promotes hyphal development and grows synergistically with *C. albicans*. They also identify a number of new target genes for further study of their roles in development of interkingdom biofilm communities.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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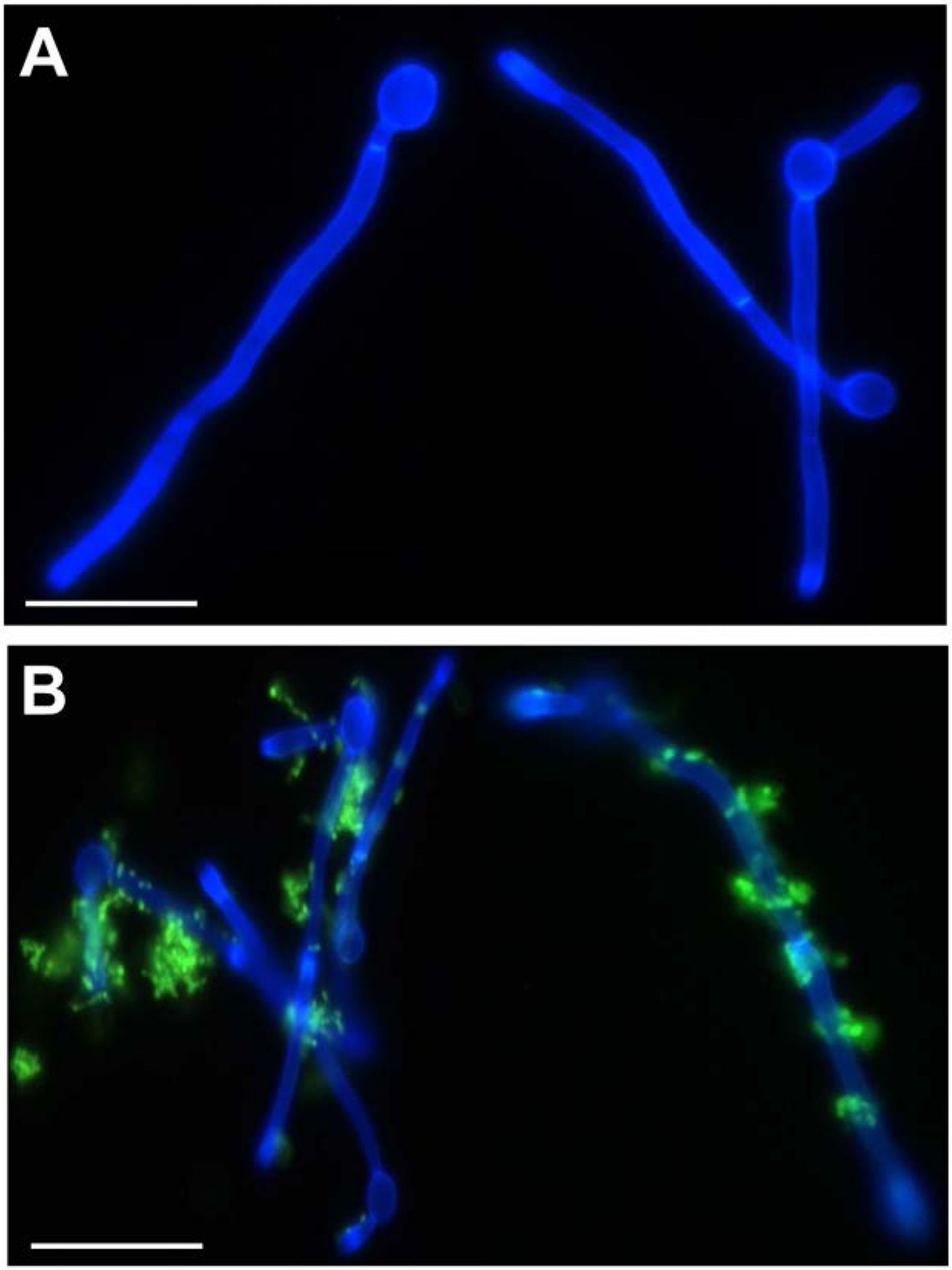

Fig. 1.
Light micrograph images of *C. albicans* SC5314 cells interacting with *S. gordonii* DL1 cells. *S. gordonii* cells were fluorescently labelled with fluorescein isothiocyanate (FITC) and incubated with filamentation-induced (2 h at 37°C) *C. albicans* for 1 h at 37°C with gentle agitation. Calcofluor white (0.3 μg ml^{-1}) was added to fluorescently label *C. albicans*. Panel A, *C. albicans* alone; Panel B, *C. albicans* and *S. gordonii* (green). Scale bar 5 μm.

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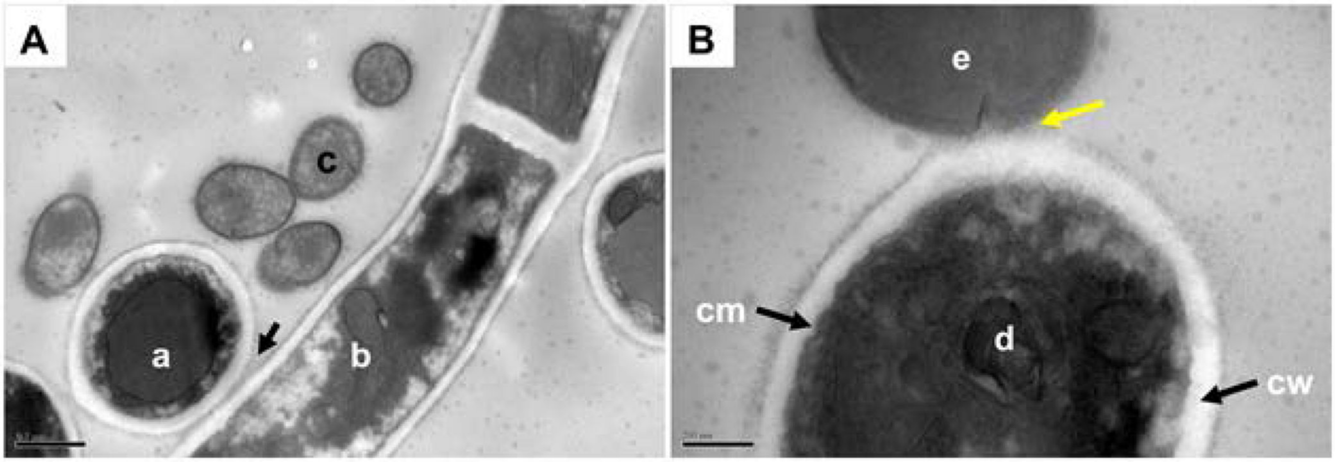

Fig. 2.

Transmission electron microscope (TEM) images of *C. albicans* SC5314 cells interacting with *S. gordonii* DL1. Filamentation-induced *C. albicans* cells in YPT-Glc were incubated with *S. gordonii* cells for 1 h at 37°C. Cells were fixed, resin embedded and sectioned (see Experimental procedures) for visualization by TEM. Panel A, vertical cross section of a *C. albicans* hyphal filament (a) shows cell wall in close contact (arrowed) with the cell wall of a longitudinally-sectioned hyphal filament (b). Smaller streptococcal cells (c) can clearly be seen nearby expressing surface fibrillar structures. Panel B, shows the cell membrane (cm) and cell wall (cw) of a vertical section of a *C. albicans* hyphal filament (d) with the cell wall physically associated with the outer wall of an *S. gordonii* cell (e). The interaction is occurring at the newly forming septum of the streptococcal cell and the fibrils appear to interdigitate with the material on the *C. albicans* cell surface (yellow arrowed). Scale bars: A, 0.5 μm; B, 200 nm.

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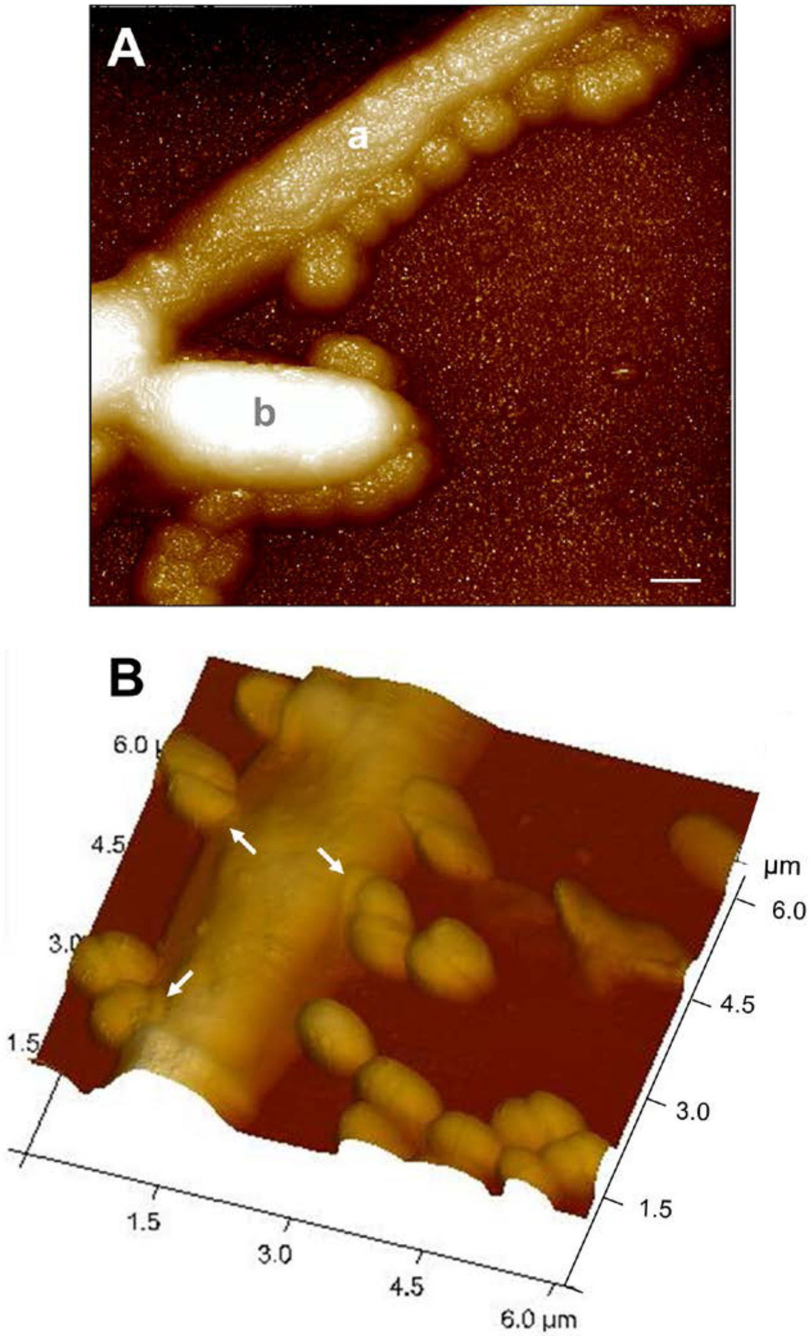

Fig. 3.

Scanning Probe Microscope (SPM) images of *C. albicans* SC5314 hypha-forming cells interacting with *S. gordonii* DL1. Filamentation-induced *C. albicans* cells in YPT-Glc medium were incubated with *S. gordonii* cells for 1 h at 37°C. Cells were then deposited onto glass cover slips and imaged in contact mode as described in Experimental procedures. Panel A, undried specimen showing hyphal filament (a) with smaller streptococcal cells attached along its length. A budding pseudohypha (b) appears also to have streptococci attached. Panel B, dried specimen showing numerous streptococcal cells in close physical contact with a *C. albicans* hyphal filament. At the point of contact there is an annular modification visible on the *C. albicans* cell surface (arrowed), implying a hyphal cell surface structural response. Note that quite often the streptococcal cell septum region was involved in binding hyphae (see also Fig. 2B). Scale bar 0.5 μm.

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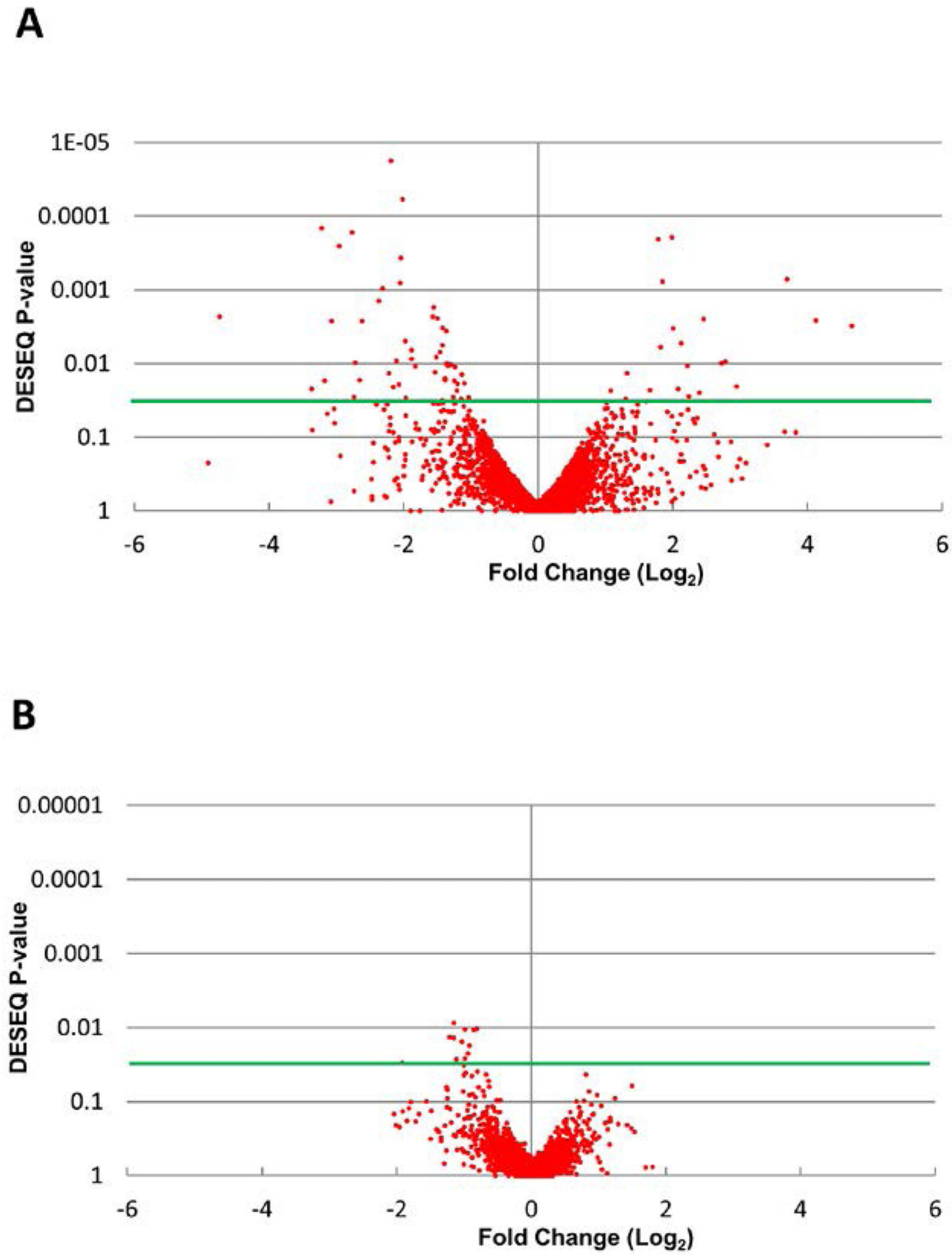

Fig. 4.

Distribution of differentially regulated genes of *C. albicans* and *S. gordonii* following co-incubation for 1 h at 37°C. RNA was extracted and gene transcriptional levels were determined following Ilumina HISeq2500 sequencing. The transcriptional profiles were constructed and analyzed using the statistical software DESeq. Volcano plots of *P*-value vs. mean fold change in gene expression were constructed for *C. albicans* genes when incubated with *S. gordonii* (A) and *S. gordonii* genes when incubated with *C. albicans* (B). Green horizontal lines represent $P = 0.05$.

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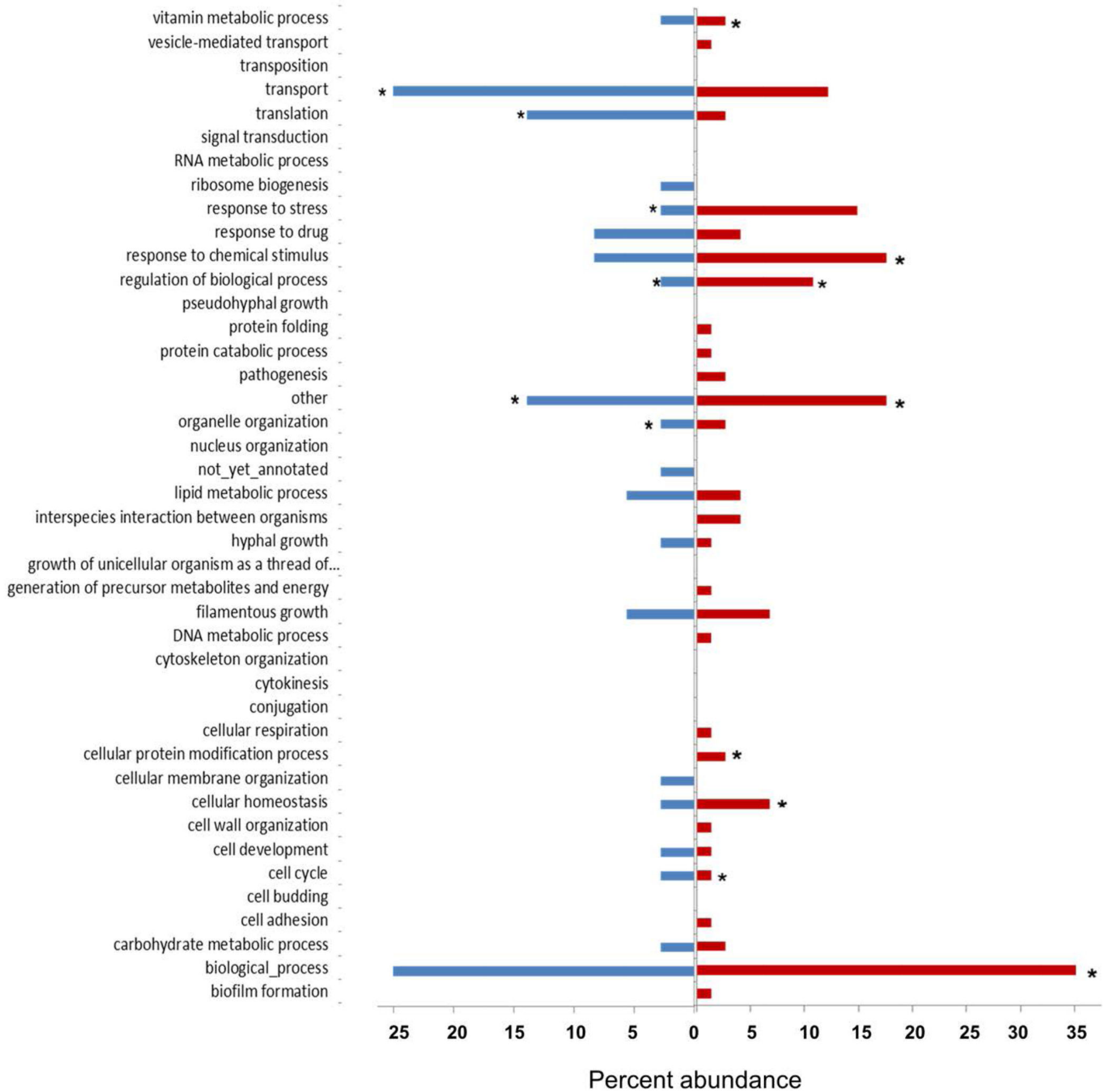

Fig. 5.

Distribution of significantly up- and down-regulated *C. albicans* genes in the Gene Ontology (GO) category Biological Process following *C. albicans* co-incubation with *S. gordonii.* Transcriptional profiles were analyzed with DESEQ. There were 75 significantly up-regulated genes, and 36 significantly down-regulated genes, which were assigned as a percentage to 41 identified level-2 GO-categories associated with the GO namespace Biological Process. *Denotes significance ($P \leq 0.05$) based on hypergeometric distribution test. Red, up-regulated; blue, down-regulated.

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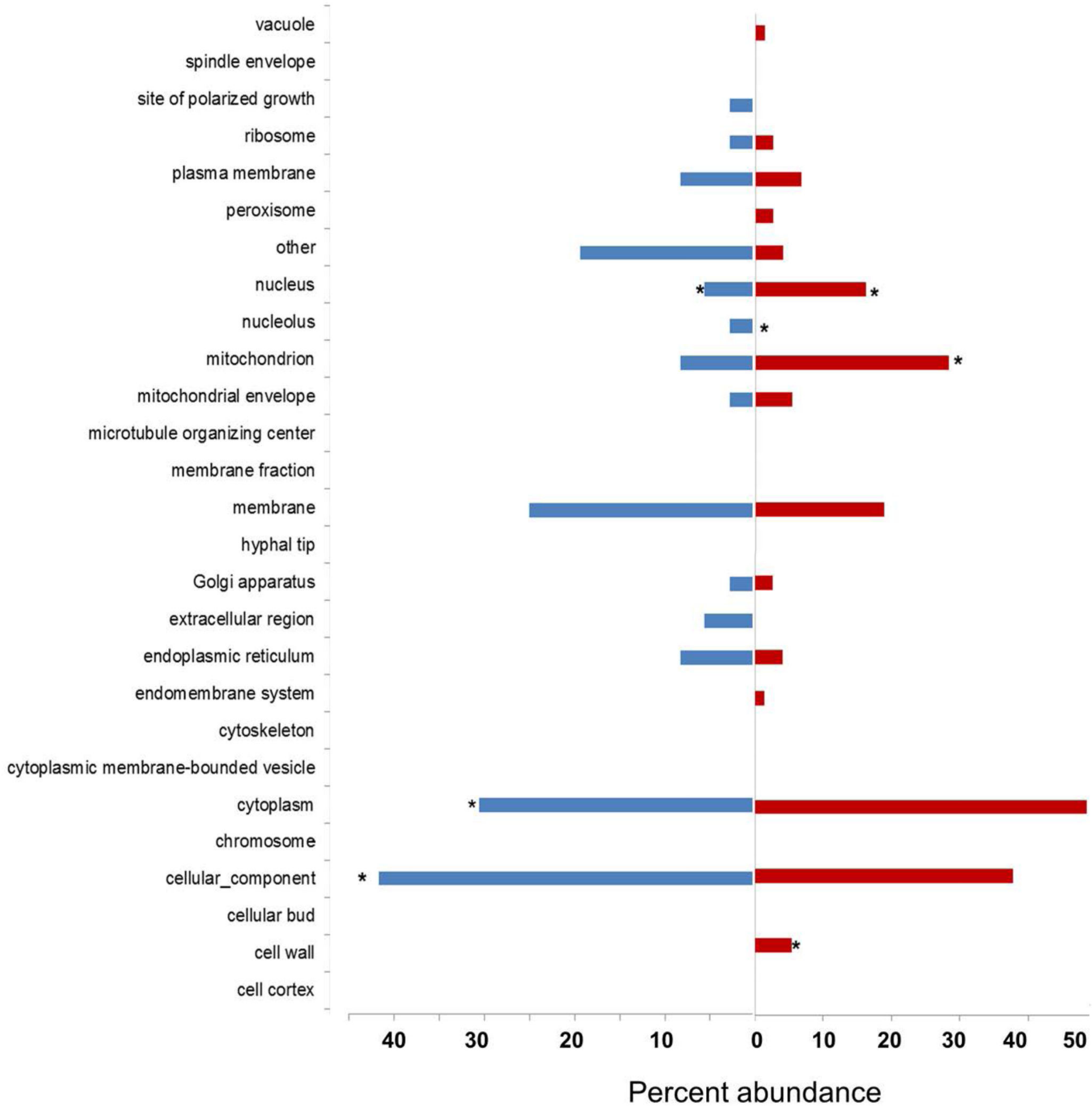

Fig. 6.

Distribution of significantly up- and down-regulated *C. albicans* genes in the Gene Ontology (GO) category Cellular Component following *C. albicans* co-incubation with *S. gordonii*. Transcriptional profiles were analyzed with DESeq. There were 75 significantly up-regulated, and 36 significantly down-regulated genes, which were assigned as a percentage to 27 level-2 GO-categories associated with the GO namespace Cellular Component. *Denotes significance ($P \leq 0.05$) based on hypergeometric distribution test.

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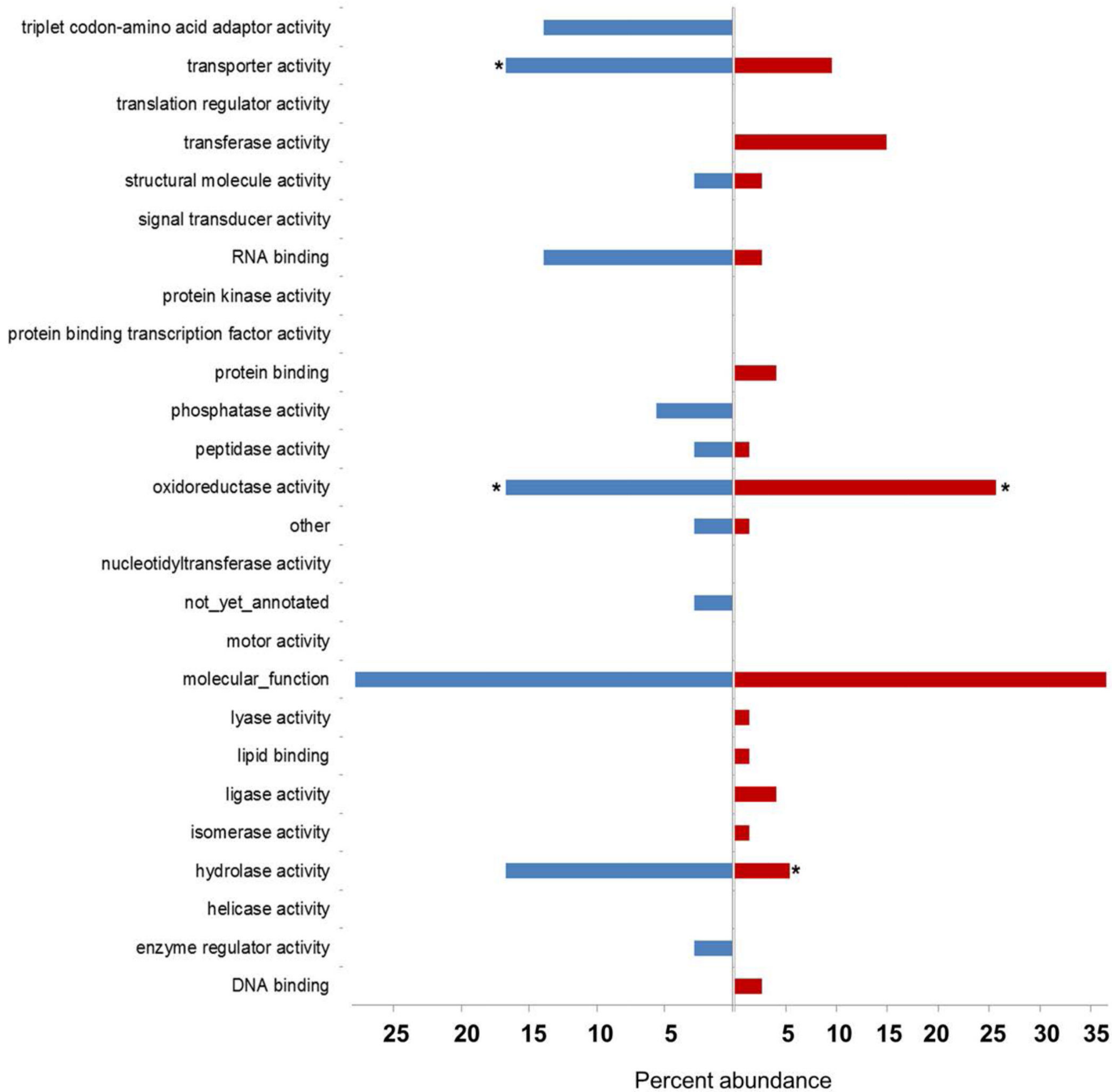

Fig. 7.

Distribution of significantly up- or down-regulated *C. albicans* genes in the Gene Ontology (GO) category Molecular Function following *C. albicans* co-incubation with *S. gordonii*. Transcriptional profiles were analyzed by the statistical software DESeq. There were 75 significantly up-regulated genes and 36 significantly down-regulated genes which were assigned as a percentage to 26 identified level-2 GO-categories associated with the GO namespace Molecular Function. *Denotes significance ($P \leq 0.05$) based on hypergeometric distribution test.

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Table 1

Composition of microbial cultures utilized to determine transcriptional changes in *C. albicans* or *S. gordonii* genes following co-incubation.

Culture no.	Conditions and components
1	*C. albicans* in YPT-Glc, 3 h at 37°C
2	*C. albicans* in YPT-Glc, 2 h at 37°C; + *S. gordonii* DL1 in YPT-Glc, 1 h at 37°C
3 [1]	*C. albicans* in YPT-Glc, 2 h at 37°C; + YPT-Glc, 1 h at 37°C
4 [2]	*C. albicans* in YPT-Glc, 2 h at 37°C, spent medium; + *S. gordonii* in YPT-Glc, 1 h at 37°C
5 [3]	*S. gordonii* in YPT-Glc, 1 h at 37°C

[1] included to account for effects of adding fresh growth medium in (2)

[2] included to account for effects of *C. albicans* spent medium on *S. gordonii*

[3] used to correct for effects of nutritional shift-down in (2)

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Table 2

List of 111 *Candida albicans* genes significantly ($P \leq 0.05$) differentially expressed following co-incubation of *C. albicans* filamentation-induced cells with *S. gordonii* for 1 h at 37°C in YPT-Glc medium.

ORF	Gene name	Fold change (log_2)[1]	Description[2]	Total abundance[3] (log_2)	*P*-value[4]
orf19.3537		5.47	Putative sulfiredoxin; biofilm-induced gene; regulated by Tsa1p, Tsa1Bp in minimal medium at 37°C	−3.310	<0.001
orf19.7469	*ARG1*	4.73	Argininosuccinate synthase; arginine biosynthesis; regulated by Gcn4p, Rim101p; induced by amino acid starvation (3-AT) and benomyl treatment; stationary phase enriched protein; repressed in alkalinizing medium; planktonic growth-induced	−2.256	0.002
orf19.3107		3.65	Ortholog of *Candida dubliniensis* CD36 : CD36_46490, *Pichia stipitis* Pignal : PICST_33598, *Candida tropicalis* MYA-3404 : CTRG_03758 and *Candida albicans* WO-1 : CAWG_03126	−3.325	<0.001
orf19.4630	*CPA1*	3.36	Putative carbamoyl-phosphate synthase subunit; alkaline down-regulated; transcription is up-regulated in both intermediate and mature biofilms	−2.646	0.022
orf19.2285		3.22	Increased transcription is observed upon benomyl treatment	−3.729	<0.001
orf19.3221	*CPA2*	3.17	Putative arginine-specific carbamoylphosphate synthetase; protein enriched in stationary phase yeast cultures; transcription is up-regulated in both intermediate and mature biofilms	−1.987	0.017
orf19.4789		3.13	Has domain(s) with predicted metal ion binding activity	−3.971	0.048
orf19.2165		3.08	Predicted ORF in Assemblies 19, 20 and 21; induced by nitric oxide	−2.750	<0.001
orf19.5610	*ARG3*	3.07	Putative ornithine carbamoyltransferase; Gcn4p-regulated; Hap43p-induced gene; repressed in alkalinizing medium	−3.019	0.003
orf19.6689	*ARG4*	3.03	Argininosuccinate lyase, catalyzes the final step in the arginine biosynthesis pathway; alkaline down-regulated; late-stage biofilm-induced	−2.027	0.042
orf19.2593	*BIO2*	2.95	Putative biotin synthase; transcriptionally up-regulated in high iron; transcription down-regulated by treatment with ciclopiroxolamine; up-regulated in clinical isolates from HIV^+ patients with oral candidiasis; Hap43p-repressed	−3.257	<0.001
orf19.176	*OPT4*	2.76	Oligopeptide transporter; detected at germ tube plasma membrane; transcriptionally induced upon phagocytosis by macrophage; fungal-specific (no human or murine homolog); Hap43p-repressed; merged with orf19.2292 in Assembly 20	−3.061	<0.001
orf19.3770	*ARG8*	2.73	Putative acetylornithine aminotransferase; Gcn2p-, Gcn4p-regulated; transcription is up-regulated in both intermediate and mature biofilms	−2.480	0.028
orf19.6229	*CAT1*	2.71	Catalase; resistance to oxidative stress, neutrophils, peroxide; role in virulence; regulated by iron, ciclopirox, fluconazole, carbon source, pH, Rim101p, Ssn6p, Hog1p, Hap43p, Sfu1p, Sef1p, farnesol, core stress response	−1.845	0.010
orf19.6899		2.65	Putative oxidoreductase; mutation confers hypersensitivity to toxic ergosterol analog	−3.382	0.017
orf19.4788	*ARG5,6*	2.61	Arginine biosynthetic enzyme activities; in *S. cerevisiae*, processed into distinct polypeptides with acetylglutamate kinase (Arg6p) activity and acetylglutamate-phosphate reductase (Arg5p) activity; Gcn4p regulated; alkaline down-regulated	−2.088	0.003

ORF	Gene name	Fold change (log_2)[1]	Description[2]	Total abundance[3] (log_2)	*P*-value[4]
orf19.3902		2.40	Predicted ORF in Assemblies 19, 20 and 21; decreased transcription is observed upon fluphenazine treatment or in an azole-resistant strain that over-expresses CDR1 and CDR2	−3.078	0.036
orf19.3395		2.36	Predicted membrane transporter, member of the drug:proton antiporter (12 spanner) (DHA1) family, major facilitator superfamily; induced by nitric oxide, oxidative stress, α-pheromone; fungal-specific; Hap43p-repressed	−3.151	0.001
orf19.5094	*BUL1*	2.31	Protein not essential for viability; macrophage/pseudohyphal-induced; similar to S. cerevisiae Bul1p, which may be involved in selection of substrates for ubiquitination	−2.361	0.001
orf19.4335	*TNA1*	2.29	Putative nicotinic acid transporter; fungal-specific (no human or murine homolog); detected at germ tube plasma membrane by mass spectrometry; transcriptionally induced upon phagocytosis by macrophage	−3.902	0.042
orf19.7042		2.25	Increased transcription is observed upon benomyl treatment or in an azole-resistant strain that over-expresses MDR1; induced by nitric oxide	−2.697	0.036
orf19.4773	*AOX2*	2.22	Alternative oxidase; induced by antimycin A, some oxidants; growth- and carbon-source-regulated; one of two isoforms (Aox1p and Aox2p); involved in cyanide-resistant respiratory pathway that is absent from S. cerevisiae; Hap43p-repressed	−2.949	0.014
orf19.4290	*TRR1*	2.19	Thioredoxin reductase; regulated by Tsa1p/Tsa1Bp, Hap43p; induced by nitric oxide, peroxide; oxidative stress-induced via Cap1p; up-regulated by human neutrophils; fungal-specific (no human/murine homolog); stationary phase enriched protein	−1.407	<0.001
orf19.125	*EBP1*	2.15	NADPH oxidoreductase; interacts with phenolic substrates such as 17β-estradiol; possible role in estrogen response; induced by oxidative, weak acid stress, nitric oxide, benomyl, GlcNAc; activated by Cap1p, Mnl1p; Sko1p; Hap43p-repressed	−2.803	0.021
orf19.6500	*ECM42*	2.10	Putative ornithine acetyltransferase; fungal-specific (no human or murine homolog); Gcn2p-, Gcn4p-regulated; clade-specific gene expression; possibly an essential gene, disruptants not obtained by UAU1 method	−2.809	0.009
orf19.113	*CIP1*	2.07	Possible oxidoreductase; transcription induced by cadmium but not by other heavy metals, heat shock, yeast-hyphal switch, oxidative stress (via Cap1p), or macrophage interaction; stationary phase enriched protein	−3.055	0.019
orf19.4689	*PGA57*	2.05	Putative GPI-anchored protein; Hap43p-induced gene	−3.348	<0.001
orf19.3131	*OYE32*	2.04	NAD(P)H oxidoreductase family protein; induced by nitric oxide, amphotericin B, oxidative stress (via Cap1p); increased transcription associated with MDR1 over-expression or benomyl treatment; macrophage-down-regulated protein abundance	−2.821	0.0003
orf19.5741	*ALS1*	2.01	Adhesin; ALS family of cell-surface glycoproteins; adhesion, virulence roles; immunoprotective; band at hyphal base; amyloid domain; biofilm-induced; Rfg1p, Ssk1p; strain background affects expression; N-term binds fucose-containing glycans	−0.998	<0.001
orf19.3981	*MAL31*	1.98	Putative high-affinity maltose transporter; transcription is up-regulated in clinical isolates from HIV+ patients with oral candidiasis; alkaline up-regulated	−2.581	0.005
orf19.5908	*TEC1*	1.97	TEA/ATTS transcription factor; involved in white cell pheromone response, regulates hypha-specific genes, biofilm formation; regulates BCR1; transcription regulated by Cph2 in some conditions; alkaline- and biofilm-induced	−2.348	0.050
orf19.2970	*LYS2*	1.96	Large subunit of heterodimeric α-aminoadipate reductase; enzyme of lysine biosynthesis; contains	−1.794	0.030

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ORF	Gene name	Fold change (log_2)[1]	Description[2]	Total abundance[3] (log_2)	*P*-value[4]
			predicted binding sites for AMP and α-aminoadipate; feedback inhibited by lysine or thialysine; regulated by Gcn2p and Gcn4p		
orf19.4873		1.89	Biofilm-induced gene; expression is regulated upon white-opaque switching	−3.192	0.009
orf19.2724		1.88	Hap43p-repressed gene; late-stage biofilm induced	−2.217	0.007
orf19.4274	*PUT1*	1.83	Putative proline oxidase; alkaline up-regulated by Rim101p; biofilm-induced	−2.623	0.011
orf19.3803	*MNN22*	1.57	Putative Golgi α-1,2-mannosyltransferase; regulated by Tsa1p, Tsa1Bp in minimal media at 37°C; Hog1p-induced; induced by nitric oxide; down-regulated in core stress response; planktonic growth-induced gene	−2.198	0.002
orf19.2833	*PGA34*	1.55	Putative GPI-anchored protein; transcription is repressed in response to α-pheromone in Spider M medium; late-stage biofilm-induced gene; induced in oropharyngeal candidiasis	−3.811	0.035
CaalfMr16	*RRNL*	1.55	Mitochondrial ribosomal RNA of the large ribosomal subunit	0.022	0.002
orf19.4513		1.53	Protein not essential for viability	−2.036	0.013
orf19.701	*CFL11*	1.51	Protein similar to ferric reductase Fre10p; flucytosine repressed; possibly adherence-induced; possibly an essential gene, disruptants not obtained by UAU1 method	−3.182	0.008
orf19.2262		1.50	Protein similar to quinone oxidoreductases; increased transcription upon benomyl treatment; induced by nitric oxide; oxidative stress-induced via Cap1p; stationary-phase enriched protein	−2.494	0.002
orf19.276		1.48	Plasma membrane-associated protein; increased transcription is observed in an azole-resistant strain that over-expresses MDR1; Hap43p-repressed gene	−2.959	0.035
orf19.7637	*YHB4*	1.46	Protein related to flavohemoglobins; not required for wild-type nitric oxide resistance; has predicted globin, FAD-binding, and NAD(P)-binding domains but lacks some conserved residues of flavohemoglobins; Hap43p-repressed gene	−2.416	0.007
orf19.1651		1.44	Ortholog of *Candida dubliniensis* CD36 : CD36_81960, *Candida tropicalis* MYA-3404 : CTRG_02378 and *Candida albicans* WO-1 : CAWG_02525	−3.581	0.045
orf19.2020	*HGT6*	1.43	Putative high-affinity major facilitator superfamily glucose transporter; 20 family members; 12 probable membrane-spanning segments; core stress response, fluconazole-induced; biofilm-induced; induced in oropharyngeal candidiasis	−1.355	0.033
orf19.7417	*TSA1*	1.43	TSA/alkyl hydroperoxide peroxidase C (AhPC) family protein; similar to thiol-dependent peroxidases of oxidative stress signaling; antigenic; hyphal surface, nucleus; yeast-form nucleus, cytoplasm; biofilm, phagocytosis, peroxide induced	−1.232	0.034
orf19.7398		1.42	Hap43p-induced gene; decreased expression in response to prostaglandins	−1.227	0.031
orf19.4482	*IFI3*	1.42	Predicted ORF in Assemblies 19, 20 and 21; constitutive expression independent of MTL or white-opaque status	−2.289	0.003
orf19.85	*GPX2*	1.42	Similar to glutathione peroxidase; expression greater in high iron; alkaline up-regulated by Rim101p; transcriptionally induced by α-factor or interaction with macrophage; regulated by Efg1p; caspofungin repressed	−2.053	0.006
orf19.2809	*CTN3*	1.41	Predicted peroxisomal carnitine acetyl transferase; Ura^+ deletion strain has no obvious metabolic, hyphal, or virulence defects; transcription induced by macrophage engulfment, hyphal growth, starvation, or non-	−2.913	0.034

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ORF	Gene name	Fold change (log_2)[1]	Description[2]	Total abundance[3] (log_2)	*P*-value[4]
			fermentable carbon sources		
orf19.7244		1.40	Putative fumarylacetoacetate hydrolase; biofilm-induced gene; induced by nitric oxide independent of Yhb1p; regulated by Sef1p, Sfu1p, and Hap43p	−2.277	0.043
orf19.6501		1.40	Ortholog of *Candida dubliniensis* CD36 : CD36_71960, *Spathaspora passalidarum* NRRL Y-27907 : SPAPADRAFT_63786, *Candida tropicalis* MYA-3404 : CTRG_05049 and *Candida albicans* WO-1 : CAWG_05564	−3.862	0.043
orf19.5760	*IHD1*	1.39	Putative GPI-anchored protein; alkaline up-regulated; greater transcription in hyphal form than yeast form; regulated by Nrg1p, Rfg1p, Tup1p; regulated by Tsa1p, Tsa1Bp in minimal media at 37°C; induced in oropharyngeal candidiasis	−1.770	0.016
orf19.5140		1.38	Ortholog of *Candida dubliniensis* CD36 : CD36_72790 and *Candida albicans* WO-1 : CAWG_05648	−3.507	0.017
orf19.1037		1.37	Ortholog of *Candida tenuis* NRRL Y-1498 : CANTEDRAFT_114303, *Candida dubliniensis* CD36 : CD36_03460, *Pichia stipitis* Pignal : PICST_63037 and *Spathaspora passalidarum* NRRL Y-27907 : SPAPADRAFT_136773	−1.996	0.004
orf19.84	*CAN3*	1.36	Hap43p-repressed gene; expression is regulated upon white-opaque switching	−2.433	0.010
orf19.4370		1.35	Predicted ORF in Assemblies 19, 20 and 21; induced by nitric oxide; oxidative stress-induced via Cap1p; fungal-specific (no human or murine homolog)	−2.730	0.010
orf19.1363		1.34	Putative protein of unknown function; late-stage biofilm-induced gene; Plc1p-regulated; transcriptionally activated by Mnl1p under weak acid stress	−2.494	0.011
orf19.3142		1.34	Ortholog of *C. parapsilosis* CDC317 : CPAR2_501210, *Candida tenuis* NRRL Y-1498 : CANTEDRAFT_97195, *Debaryomyces hansenii* CBS767 : DEHA2E08800g and *Candida dubliniensis* CD36 : CD36_46140	−3.388	0.045
orf19.238	*CCP1*	1.30	Similar to cytochrome-c peroxidase N terminus; negatively regulated by Rim101p or alkaline pH; transcription induced by interaction with macrophage or low iron; oxygen-induced activity; regulated by Sef1p, Sfu1p, and Hap43p	−1.804	0.045
orf19.3122.2		1.29	Ortholog(s) have role in exocytosis, filamentous growth, mRNA export from nucleus, proteasomal ubiquitin-dependent protein catabolic process, proteasome assembly, regulation of cell cycle	−2.692	0.010
orf19.6994	*BAT22*	1.26	Putative branched chain amino acid aminotransferase; regulated by Gcn4p; induced by farnesol treatment, GlcNAc, amino acid starvation (3-aminotriazole treatment); present in exponential and stationary growth phase yeast cultures	−2.006	0.028
orf19.5762	*PGA61*	1.26	Putative GPI-anchored protein	−3.810	0.044
orf19.699		1.25	Biofilm-induced gene	−2.757	0.035
orf19.690	*PLB2*	1.25	Putative phospholipase B; conserved catalytic region; 6 putative *N*-glycosylation motifs; predicted secretion signal; no GPI anchor predicted; fungal-specific (no human or murine homolog)	−3.009	0.017
orf19.3443	*OYE2*	1.24	Putative NAPDH dehydrogenase; fungal-specific (no human or murine homolog); induced by nitric oxide	−2.530	0.011
orf19.4653		1.24	Transcriptionally regulated by iron; expression greater in low iron; similar to GPI-linked cell-wall proteins	−2.937	0.034
orf19.3528		1.21	Ortholog(s) have cytosol, nucleus localization	−3.001	0.023

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ORF	Gene name	Fold change (log_2)[1]	Description[2]	Total abundance[3] (log_2)	*P*-value[4]
orf19.847	*YIM1*	1.20	Protein similar to protease of mitochondrial inner membrane; increased transcription is observed upon benomyl treatment; macrophage-down-regulated gene	−2.193	0.019
orf19.1077	*ATM1*	1.15	Member of MDR subfamily of ABC family; similar to *S. cerevisiae* ABC transporter, Atm1p; transcriptionally regulated by iron; expression greater in low iron; induced by nitric oxide independent of Yhb1p	−2.405	0.030
CaalfMr17	*RRNS*	1.13	Mitochondrial ribosomal RNA of the small ribosomal subunit	0.567	0.014
orf19.1701	*RKI1*	1.11	Ortholog(s) have ribose-5-phosphate isomerase activity, role in pentose-phosphate shunt, pyridoxine biosynthetic process and cytoplasm, nucleus localization	−2.653	0.038
orf19.6947	*GTT11*	1.10	Glutathione S-transferase, localized to ER; induced in exponentially growing cells, under oxidative stress; induced by nitric oxide	−2.348	0.019
orf19.4147	*GLR1*	1.03	Glutathione reductase; up-regulated by human neutrophils; oxidative stress-induced regulation via Cap1p; over-expression correlates with multidrug resistance in a *cap1* mutant, farnesol induced; stationary phase enriched protein	−1.585	0.029
orf19.7567		1.03	Predicted ORF in Assemblies 19, 20 and 21; transcription is induced in response to α-pheromone in Spider M medium	−2.655	0.043
orf19.2179.2	*RPS10*	−0.99	Ribosomal protein S10; down-regulated in the presence of human whole blood or polymorphonuclear (PMN) cells	−1.674	0.041
orf19.54	*RHD1*	−1.01	Putative β-mannosyltransferase, required for the addition of β-mannose to the acid-labile fraction of cell wall phosphopeptidomannan; member of a 9-gene family; transcriptionally regulated on yeast-hyphal and white-opaque switches	−1.871	0.034
orf19.1860.1		−1.03	Has domain(s) with predicted aminopeptidase activity and role in proteolysis	−1.689	0.041
orf19.5839	*PDR17*	−1.08	Fungal-specific protein (no human or murine homolog); role in sensitivity to fluconazole, specifically	−2.608	0.023
orf19.1264	*CFL2*	−1.08	Putative oxidoreductase, iron utilization; regulated by Sfu1p, Sef1p, Hap43p, Nrg1p, Tup1p, Rim101p; alkaline, low iron, fluphenazine, ciclopirox olamine, flucytosine, fluconazole, biofilm induced; caspofungin, amphotericin B repressed	−1.512	0.047
orf19.252		−1.09	Putative protein of unknown function; Hap43p-repressed gene; *S. cerevisiae* ortholog FMP37 localizes to mitochondria	−2.420	0.047
orf19.4151	*SPO1*	−1.25	Protein similar to phospholipase B; fungal-specific (no human or murine homolog)	−3.236	0.042
orf19.5673	*OPT7*	−1.25	Putative oligopeptide transporter; possibly transports GSH or related compounds; induced by biofilm formation; Hog1p-induced; expression of *OPT6*, *-7*, or *-8* does not suppress defect of mutant lacking *OPT1-3*; fungal-specific; Hap43p-repressed	−2.516	0.049
orf19.1258		−1.30	Putative adhesin-like protein; regulated by Tsa1p, Tsa1Bp in minimal media at 37°C; transcription is induced in response to alpha pheromone in Spider M medium; clade-associated gene expression; Hap43p-induced gene	−2.537	0.030
orf19.5806	*ALD5*	−1.32	NAD-aldehyde dehydrogenase; decreased expression in fluconazole-resistant isolate, or in hyphae; biofilm induced; fluconazole-down-regulated; protein abundance is affected by *URA3* expression in the CAI-4 strain; stationary phase enriched	−0.500	0.014

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ORF	Gene name	Fold change (log_2)[1]	Description[2]	Total abundance[3] (log_2)	*P*-value[4]
snR61		−1.40	C/D box small nucleolar RNA (snoRNA)	−3.152	0.045
orf19.5986	*THI4*	−1.48	Thiamine biosynthetic enzyme precursor; repressed during the mating process; fungal-specific (no human or murine homolog); stationary phase enriched protein; planktonic growth-induced gene	−3.220	0.046
orf19.2619	*PHO113*	−1.48	Putative constitutive acid phosphatase; transcription is negatively regulated by Rim101p; DTT-extractable; *N*-glycosylated; possibly an essential gene, disruptants not obtained by UAU1 method	−2.760	0.036
orf19.1608		−1.61	Ortholog(s) have cytosol, nucleus localization	−2.858	0.032
orf19.984	*PHO8*	−1.66	Putative repressible vacuolar alkaline phosphatase; transcription is positively regulated by Rim101p; regulated by Tsa1p, Tsa1Bp in minimal media at 37°C; possibly adherence-induced	−3.745	0.023
orf19.1263	*CFL1*	−1.78	Protein similar to ferric reductase Fre10p; possible functional homolog of *S. cerevisiae* Fre1p (reports differ); transcription is negatively regulated by Sfu1p, copper, amphotericin B, caspofungin; induced by ciclopirox olamine	−1.393	<0.001
orf19.6570	*NUP*	−1.82	Nucleoside permease; adenosine and guanosine are substrates, whereas cytidine, adenine, guanine, uridine, uracil are not; similar to a nucleoside permease of *S. pombe*; possibly processed by Kex2p	−2.942	0.006
orf19.3934	*CAR1*	−1.85	Arginase involved in arginine catabolism; transcription regulated by Nrg1p, Mig1p, and Tup1p; colony morphology-related regulation by Ssn6p; alkaline up-regulated; protein decreased in stationary phase; biofilm-induced; sumoylation target	−1.998	<0.001
tE(UUC)4	*tE(UUC)4*	−1.92	tRNA-Glu, predicted by tRNAscan-SE; UUC anticodon	−3.276	0.046
orf19.4211	*FET3*	−1.98	Multicopper oxidase; required for growth in low-iron; required for prostaglandin E2 production; functional homolog of *S. cerevisiae* Fet3p; ketoconazole, caspofungin, amphotericin B repressed; regulated by Sef1p, Sfu1p, and Hap43p	−0.909	<0.001
RDN18	*RDN18*	−2.01	18S ribosomal RNA; component of the small (40S) ribosomal subunit; encoded in about 55 copies of the rDNA repeat on Chromosome R	−0.717	0.003
tI(AAU)2	*tI(AAU)2*	−2.07	tRNA-Ile, predicted by tRNAscan-SE; AAU anticodon	−3.416	0.022
snR57b		−2.12	C/D box small nucleolar RNA (snoRNA)	−3.210	0.005
tE(UUC)6	*tE(UUC)6*	−2.12	tRNA-Glu, predicted by tRNAscan-SE; UUC anticodon	−3.135	0.050
orf19.3152	*AMO2*	−2.21	Protein similar to *A. niger* predicted peroxisomal copper amino oxidase; mutation confers hypersensitivity to toxic ergosterol analog; induced upon biofilm formation	−1.986	0.011
orf19.6520		−2.22	Putative allantoate permease; fungal-specific (no human or murine homolog)	−3.636	0.042
tE(UUC)3	*tE(UUC)3*	−2.24	tRNA-Glu, predicted by tRNAscan-SE; UUC anticodon	−3.269	0.028
snR69		−2.40	C/D box small nucleolar RNA (snoRNA)	−3.517	0.025
orf19.2652	*TEF4*	−2.46	Putative translation elongation factor; genes encoding ribosomal subunits, translation factors, and tRNA synthetases are down-regulated upon phagocytosis by murine macrophage	−3.230	0.003
orf19.7071	*FGR2*	−2.73	Protein similar to phosphate transporters; transposon mutation affects filamentous growth; expression is regulated upon white-opaque switching	−3.598	0.010

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ORF	Gene name	Fold change (log_2)[1]	Description[2]	Total abundance[3] (log_2)	*P*-value[4]
snR67		−2.78	C/D box small nucleolar RNA (snoRNA)	−3.508	0.009
orf19.2197		−2.95	Has domain(s) with predicted N,N-dimethylaniline monooxygenase activity, NADP binding, flavin adenine dinucleotide binding activity and role in oxidation-reduction process	−3.766	0.020
orf19.4599	*PHO89*	−3.70	Putative phosphate permease; expression is regulated upon white-opaque switching; alkaline up-regulated by Rim101p; induced upon biofilm formation; possibly adherence-induced	−2.523	0.001
orf19.1344		−4.12	Predicted ORF in Assemblies 19, 20 and 21; fluconazole-induced	−2.533	0.003
tL(UAA)3	*tL(UAA)3*	−4.66	tRNA-Leu, predicted by tRNAscan-SE; UAA anticodon	−3.785	0.003
xi-Rb		Inf	Long terminal repeat (LTR); about 387 bp long, 5 copies per genome	−4.329	0.029

[1] Positive values, up-regulated; negative values, down-regulated ($log_2 \geq 1.0$ equivalent to ≥ twofold linear change)

[2] Description details from the CGD (www.candidagenome.org)

[3] Abundance reads represent the fractional expression of all targets in the genome

[4] *P*-values were calculated from DESeq and adjusted *P*-values ≤ 0.050 were considered to be significant

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Table 3

Mean fold changes in transcription levels of genes associated with *C. albicans* filamentous growth and pathogenesis following co-incubation with *S. gordonii*.

Gene*	Filamentous growth (G) or pathogenesis (P)	Mean fold change ($\log_2$)[1]	Protein identity or function[2]	Total abundance ($\log_{10}$)[3]	*P*-value[4]
FGR42	G	2.94	Protein lacking an orthologue in *S. cerevisiae*; transposon mutation affects filamentous growth	−1.941	0.181
CAT1	G/P	**2.71**	Catalase; resistance to oxidative stress	−1.845	**0.010** *
ALS1	G/P	**2.00**	Cell surface glycoprotein ALS family; biofilm formation, germ tube induction; expressed at infection of human buccal epithelial cells; GPI-modified; induced by ketoconazole, low iron and at cell wall regeneration; regulated by Sfu1	−0.998	<**0.001***
TEC1	G/P	**1.97**	TEA/ATTS transcription factor; involved in white cell pheromone response, regulates hypha-specific genes, biofilm formation; regulates *BCR1*; transcription regulated by Cph2 in some conditions; alkaline- and biofilm-induced	−2.348	**0.050** *
CUP9	G	1.55	Gene up-regulated in clinical isolates from HIV patients with oral candidiasis; transcription reduced in yeast-hyphal switch	−1.331	0.205
CAT8	G	1.52	Predicted zinc finger protein B not essential for viability	−2.687	0.152
GAL10	G	1.49	UDP-glucose 4-epimerase required for galactose utilization	−1.941	0.269
HSP21	G/P	1.46	Small heat shock protein, stress response and virulence	−2.800	0.374
TSA1	G	**1.43**	TSA/alkyl hydroperoxide peroxidase C (AhPC) family protein; similar to thiol-dependent peroxidases of oxidative stress signaling; antigenic; hyphal surface; peroxide induced	−1.232	**0.034** *
CTN3	G	**1.41**	Predicted peroxisomal carnitine acetyl transferase	−2.913	**0.034** *
MDR1	P	1.38	Stationary phase enriched protein; Gcn4-regulated;	−2.454	0.220
YHB1	G/P	1.36	Member of MDR subfamily of ABC family; similar to *S. cerevisiae* ABC transporter Atm1; transcriptionally regulated by iron; expression greater in low iron; induced by nitric oxide independent of Yhb1	−1.881	0.365
VPS20	G	1.15	ESCRT III complex protein with a role in multivesicular body (MVB) trafficking; required for processing of Rim8	−2.608	0.078
NRG2	G	1.15	Protein similar to *S. cerevisiae* Nrg2 transcription factor, which regulates invasive growth in *S. cerevisiae*; transposon mutation affects filamentous growth	−4.503	0.598
PHHB	G	1.04	Transposon mutation affects	−2.771	0.413

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Gene*	**Filamentous growth (G) or pathogenesis (P)**	**Mean fold change (log_2)[1]**	**Protein identity or function[2]**	**Total abundance (log_{10})[3]**	***P*-value[4]**
			filamentous growth; late stage biofilm-induced gene		
ICL1	P	1.03	Isocitrate lyase enzyme of glyoxylate cycle	−2.034	0.176
TTR1	P	1.02	Putative glutaredoxin; described as a glutathione reductase; up-regulated in the presence of human neutrophils, and upon benomyl treatment; alkaline down-regulated; regulated by Gcn2 and Gcn4; required for virulence in mouse model	−2.719	0.475
GAP1	G	1.00	Amino acid permease	−2.191	0.215
CHT2	G	−1.05	GPI-linked chitinase required for normal filamentous growth	−1.457	0.498
SOD5	P	−1.26	Copper- and zinc-containing superoxide dismutase; member of a gene family that includes *SOD1*, *SOD4*, *SOD5*, and *SOD6*; gene may contain an intron; Hap43-repressed; biofilm-induced	−2.507	0.329
FGR12	G	−1.41	Protein lacking an orthologue in *S. cerevisiae*; transposon mutation affects filamentous growth; biofilm induced gene	−4.642	0.590
LIP2	P	−1.69	Secreted lipase member of a differentially expressed lipase gene family	−4.418	0.486
FGR2	G	**− 2.73**	Transcription factor with zinc cluster DNA-binding motif	−3.598	**0.010** *

[1]Positive values, up-regulated; negative values, down-regulated (log_2 ≥1.0 equivalent to ≥ twofold linear change)

[2]Information from CGD (www.candidagenome.org)

[3]Abundance reads represent the fractional expression of all targets in the genome. The most highly-expressed genes on this Table are therefore (in order): *ALS1*, *TSA1*, *CUP9* and *CHT2*

[4]*P*-values were calculated from DESeq and adjusted *P*-values ≤ 0.05 were considered to be significant

*Bold type significant change *P* ≤0.05

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Table 4

Mean fold changes in expression of *C. albicans* genes associated with adherence, or encoding covalently-linked cell wall proteins or GPI-modified proteins, following co-incubation with *S. gordonii*.

Gene*	**Association**[1]	**Mean fold change ($\log_2$)**[2]	**Total abundance ($\log_{10}$)**[3]	***P*-value**[4]
PGA10	CL, GPI	2.23	−3.196	0.147
PGA37	GPI	2.20	−3.680	0.054
PGA57	GPI	**2.05**	−3.348	<**0.001***
ALS1	CL, GPI, Adh	**2.01**	−0.998	<**0.001***
TEC1	Adh	**1.97**	−2.348	**0.050** *
HIS4	Adh	1.58	−1.663	0.160
PGA34	GPI	**1.55**	−3.811	**0.035** *
PGA60	GPI	1.49	−3.813	0.083
PGA36/IHD1	GPI	**1.39**	−1.172	**0.016** *
EAP1	CL, Adh	1.39	−3.295	0.062
PGA61	GPI	**1.26**	−3.810	**0.044** *
ORF19.4653	GPI	**1.24**	−2.256	**0.034** *
HYR1	CL, GPI, Adh	1.10	−2.300	0.253
CHT2	CL, GPI	−1.05	−1.457	0.498
PGA3/SOD5	CL, GPI	−1.26	−2.507	0.329
PGA16	GPI	−1.42	−5.187	0.959
CSA1	CL, GPI	−1.43	−1.638	0.280
PGA39	GPI	−1.56	−4.541	0.487

[1] Adh, adhesion; CL, covalently linked; GPI, Glycophosphatidylinositol-modified

[2] Positive values, up-regulated; negative values, down-regulated ($\log_2 \geq 1.0$ equivalent to ≥ twofold linear change)

[3] Abundance reads represent the fractional expression of all targets in the genome

[4] *P*-values were calculated from DESeq and adjusted *P*-values ≤ 0.05 were considered to be significant

[*] Bold type significant change *P* ≤0.05

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Table 5

Mean fold changes in expression of *S. gordonii* genes following co-incubation with *C. albicans*.

Gene*	Mean fold change (log_2)[1]	Description[2]	Total abundance (log_{10})[3]	*P*-value[4]
glpK	**1.92**	Glycerol kinase	−1.612	**0.030** *
SGO_0149	1.91	Hypothetical protein	−2.812	0.135
SGO_0092	1.85	Hypothetical protein	0.000	0.181
SGO_0093	1.81	Hypothetical protein	0.000	0.123
SGO_0631	1.78	α-glyerophosphae oxidase	−0.955	0.101
SGO_1071	1.67	Hypothetical protein	−0.961	0.146
adk	1.56	Adenylate kinase	−1.587	0.100
xpt	1.49	Xanthine phosphoribosyltransferase	−1.144	0.318
rpoA	1.48	DNA-directed RNA polymerase	−0.282	0.133
SGO_2091	1.37	Hypothetical proein	−2.210	0.257
SGO_2079	1.29	Hypothetical protein	0.000	0.690
rplR	1.26	50S ribosomal protein	−0.776	0.064
SGO_1136	1.25	Esterase superfamily protein	−2.636	0.119
SGO_1581	1.25	MtN3/saliva family protein	−2.511	0.091
rpsS	1.24	30S ribosomal protein S19	−1.088	0.069
rplO	**1.21**	Ribosomal protein	−1.086	**0.014** *
trpA-1	1.18	Tryptophan synthase subunit α	0.000	0.193
celB	**1.15**	Cellobiose metabolism operon	−1.538	**0.014** *
rplN	**1.15**	Ribosomal protein L14	−0.918	**0.009** *
rplB	**1.11**	Ribosomal protein L2	−0.599	**0.027** *
SGO_0630	1.05	Glycerol uptake facilitator protein	−1.642	0.256
rpsE	**1.02**	Ribosomal protein S5	−0.666	**0.016** *
ciaR	**1.00**	Transcription regulator	−0.885	**0.032** *
SGO_1261 (gat)	**1.00**	Glutamine amidotransferase	−1.765	**0.044** *
rpmD	**0.98**	Ribosomal protein L30	−0.961	**0.011** *
celA	**0.98**	Cellobiose metabolism operon	−0.738	**0.026** *
SGO_0571	**0.97**	Hypothetical protein	0.000	**0.041** *
rplX	**0.94**	Ribosomal protein L24	−1.020	**0.023** *
secY	**0.92**	Preprotein translocase subunit	−1.095	**0.018** *
SGO_1625	**0.88**	Acetoin utilization	−2.511	**0.045** *
SGO_1579	**0.85**	Transcription antiterminator (BglG family)	−0.745	**0.011** *
celD	**0.80**	Cellobiose metabolism operon	−0.775	**0.010** *

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Gene*	**Mean fold change (log$_2$)[1]**	**Description[2]**	**Total abundance (log$_{10}$)[3]**	***P*-value[4]**
rplF	**0.80**	Ribosomal protein L6	−1.062	**0.039** *
celC	**0.67**	Cellobiose metabolism operon	−0.989	**0.043** *
SGO_0991	−0.31	Hypothetical protein	−2.812	1.000
SGO_0924	−0.53	HsdD	0.000	0.921
sodA	−0.57	Superoxide dismutase	−0.818	0.760
SGO_1461	−0.62	Stress response transcriptional regulator	−2.511	0.630
SGO_0378	−0.64	Hypthothetical protein	−0.567	0.783
rpmF	−0.73	Ribosomal protein L32	−1.409	0.650
rpmG	−0.79	Ribosomal protein L33	−2.636	0.403
glgP-2	− **0.82**	Maltodextrin phosphorylase	−0.913	**0.043** *
trx-2	−0.83	Thioredoxin 2	−2.113	0.201
gloA	−0.83	Lactolylglutathione lyase	−1.454	0.347
hutU	−0.85	Urocanate hydratase	−2.414	0.235
SGO_0584	−0.85	Acetyltransferase	−1.591	0.073
SGO_2084	−0.86	Quinone family NAD(P)H deydrogenase	0.000	0.406
ldh	−0.89	Lactate dehydrogenase	−0.791	0.111
SGO_1457	−0.90	Hypothetical protein	−2.210	0.367
SGO_1300	−0.90	NADPH-dependant reductase	−2.210	0.465
pyrB	−0.91	Aspartate carbomyl transferase	−1.260	0.295
gtfG	−0.91	Glucosyltransferase	−0.943	0.151
SGO_1301	−0.93	Hypothetical protein	−2.812	0.322
SGO_0194	−0.94	Hypothetical protein	−1.958	0.265
ftcD	−0.96	Glutamate formiminotransferase	0.000	0.323
SGO_0233	−0.98	Lipoprotein	0.000	0.083
SGO_1740	−0.99	Integral membrane protein	−1.834	0.575
bta	−1.01	Bacteriocin transport accessory protein	−1.334	0.300
SGO_1508	−1.01	Uncharacterized	0.000	0.650
secE	−1.04	Preprotein translocase subunit	−2.636	0.113
mscL	−1.10	Mechanosensitive channel protein	−1.312	0.196
SGO_1256	−1.13	PyrK(dihydroorotate dehydrogenase)	−2.812	0.238
SGO_1108	−1.15	Uracil permease	−0.812	0.187
SGO_1106	−1.16	Hypthothetical protein	−1.958	0.384
trx-1	−1.17	Thioredoxin 1	−0.957	0.164
SGO_1107	−1.18	PyrR (pyrimidine regulatory protein)	−2.812	0.182
glmS	−1.25	Glucosamine fructose-6-phosphate aminotransferase	−1.001	0.090
pyrd	−1.29	Dihydroorotate dehydrogenase	−3.113	0.202

Gene*	Mean fold change (log_2)[1]	Description[2]	Total abundance (log_{10})[3]	*P*-value[4]
carB	−1.42	Carbamyl phosphate synthetase	−0.291	0.207
pyrE	−1.49	Orotate phosphoribosyl transferase	−2.636	0.228
SGO_0296	−1.50	Integral membrane protein	0.000	0.061
SGO_1105	−1.54	Hypothetical protein	−1.958	0.257

[1] Positive values, up-regulated; negative values, down-regulated (log_2 ≥1.0 equivalent to ≥ twofold linear change)

[2] Description details from the *S. gordonii* CH1 annotated genome sequence

[3] Abundance reads represent the fractional expression of all targets in the genome

[4] *P*-values were calculated from DESeq and adjusted *P*-values ≤ 0.05 were considered to be significant

* Bold type significant change *P* ≤0.05

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