Transcriptional Profiles of the Response to Ketoconazole and Amphotericin B in *Trichophyton rubrum*

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*Trichophyton rubrum* (*T. rubrum*) is a pathogenic filamentous fungus of increasing medical concern. Two antifungal agents, ketoconazole (KTC) and amphotericin B (AMB), have specific activity against dermatophytes. To identify the mechanisms of action of KTC and AMB against *T. rubrum*, a cDNA microarray was constructed from the expressed sequence tags (ESTs) of the cDNA library from different developmental stages, and transcriptional profiles of the responses to KTC and AMB were determined. *T. rubrum* was exposed to sub-inhibitory concentrations of KTC and AMB for 12 hours, and microarray analysis was used to examine gene transcription. KTC exposure induced transcription of genes involved in lipid, fatty acid, and sterol metabolism,
including ERG11, ERG3, ERG25, ERG6, ERG26, ERG4, CPO, INO1, DW700960, CPR, DW696584, DW406350, and ATG15. KTC also increased transcription of the multidrug resistance gene ABC1. AMB exposure increased transcription of genes involved in lipid, fatty acid, and sterol metabolism (DW696584, EB801458, IVD, DW694010, DW688343, DW684992), membrane transport (Git1, DW706156, DW684040, DMT, DW406136, CCH1, DW710650) and stress-related responses (HSP70, HSP104, GSS, AOX, EB801455, EB801702, TDH1, UBI4), but reduced transcription of genes involved in maintenance of cell-wall integrity and signal transduction pathways (FKS1, SUN4, DW699324, GAS1, DW681613, SPS1, DW703091, STE7, DW703091, DW695308) and some ribosomal proteins. This is the first report of the use of microarray analysis to determine the effects of drug action in T. rubrum.

Dermatophytosis is a common disease that can affect a large proportion of the population (36). The incidence of dermatophytoses has increased over recent years, particularly in immunocompromised patients (8, 29, 31). The dimorphic anthropophilic fungus Trichophyton rubrum is an important cause of superficial dermatomycoses such as onychomycosis and tinea pedis (15, 39), and is known to account for as many as 69.5% of all dermatophyte infections. T. rubrum infections are often intractable, and relapse frequently occurs after cessation of antifungal therapy (20).

Theazole antifungal agents such as itraconazole, clotrimazole, and ketoconazole are generally used to treat dermatomycosis (9, 22, 30). The mechanism of action of azoles involves inhibiting the cytochrome P450 enzyme lanosterol demethylase by binding to the
heme in the active site of the enzyme (26). Amphotericin B (AMB) is a type of polyene antifungal drug, which binds to ergosterol in the fungal cell membrane, thereby compromising membrane integrity and ultimately leading to cell death (10). AMB is not effective in the treatment of dermatophyte infections (27), but has been shown to be very active against *T. rubrum in vitro* (7). The difference between the antifungal activity of AMB against *T. rubrum in vivo* and *in vitro* is a reason to determine its mechanism of action against this pathogenic fungus. cDNA microarrays are a good tool for drug target identification as they can survey the global effects mediated by the addition of antifungal agents. Although the mechanisms of action of antifungal agents against some model fungi such as *Saccharomyces cerevisiae* and *Candida albicans* have been studied by cDNA microarrays (1, 3, 17), the inhibitory mechanisms of KTC and AMB against *T. rubrum* are still poorly understood.

To establish a molecular base for understanding the biological function of *T. rubrum*, we created *T. rubrum* cDNA library derived from various developmental stages. The *T. rubrum* genome was estimated to be 22.05 Mb (6). We obtained 11,085 first-pass unique ESTs and used sequence analysis and database searching to identify known genes, define putative novel genes, and to propose some possible metabolic networks in this fungus. To identify class-specific and mechanism-independent changes in gene expression, we examined changes in transcriptional profiles of *T. rubrum* in response to the azole and polyene classes of antifungal agents by cDNA microarray.

As mentioned above, the prevalence of *T. rubrum* infections and its anthropophilic nature make it a good model for the study of human pathogenic filamentous fungus, and our work in
identifying ESTs in \textit{T. rubrum} cDNA libraries will improve our understanding of the molecular mechanisms of its growth, metabolism, pathogenesis and drug resistance.

**EXPERIMENTAL PROCEDURES**

**Fungus and Material.** The \textit{T. rubrum} clinical isolate BMU 01672 used in this study was provided by Professor Ruoyu Li (Research Center for Medical Mycology, Peking University). The isolate was confirmed as \textit{T. rubrum} by morphologic identification of both microscopic and macroscopic characteristics (5, 16), as well as by PCR amplification and sequencing of the 18S ribosomal DNA and internal transcribed spacer (ITS) regions (13). The potato dextrose agar (PDA), yeast extract, peptone and D-glucose used for the strain cultures were bought from Difco.

**Antifungal agents.** KTC and AMB were obtained from Sigma (St. Louis, MO). Stock solutions of varying concentrations were made in dimethyl sulfoxide (DMSO; Sigma).

**Preparation of cell cultures.** Potato dextrose agar (39 g/L) was inoculated with a few hyphae of \textit{T. rubrum} and incubated at 28°C for 2–3 weeks until good conidiation was produced. The mixture of conidia and hyphal fragments were collected in distilled water. The conidia, sprouted conidia and mycelia of different developmental stages to be used for construction of cDNA library were collected as follows: (i) the conidia were harvested by Whatman’s filter model 40, (ii) a group of conidia were incubated at 28°C in YPG medium (10 g/L yeast extract, 20 g/L peptone, 10 g/L D-glucose) for approximately 8 hours to
produce sprouted conidia, and (iii) the hyphae were placed in 100-mL aliquots of YPG medium and incubated in a 28°C bath shaker for 7, 10, 14, 15, 16, 20, 22, 26, 28, 34 and 36 days. The above-mentioned collections were centrifuged respectively, the supernatant was discarded and the pelleted material was washed twice with PBS.

**Construction of cDNA Libraries and Sequencing.** The cDNA libraries were constructed following the protocols of the SUPERSCRIPT™ Plasmid System with GATEWAY™ Technology for cDNA Synthesis and Cloning (Invitrogen). The cDNA plasmids were isolated by the Millipore method using MADV filter plates. Sequencing was performed with a generic T7 primer located 5’ upstream of the inserted segments, following the protocol of the PRISM Big Dye Terminator Kit on an ABI3700 automated sequencer.

**EST Processing Pipeline and Annotation.** Phred quality assessment and computational analysis were carried out as follows. The trace files from the sequencer were ‘basecalled’ by Phred with the quality value set at >Q15. The cross-match software from the Phrap package (http://www.phrap.org) was used to remove vector sequences. The polyA tails were clipped from some of the ESTs using the Trimmest program from the EMBOSS package. Processed ESTs longer than 200 bp were clustered by comparing all base pairs using BLASTN, and collecting those with >95% identity over regions longer than 40 bp and with unmatched overhangs <20 bp. The sequences contained in each cluster were assembled using CAP3 to identify the consensus ESTs. The BLASTX program (2) was used for annotation, together with GenBank™ nonredundant (nr) clusters of orthologous groups (COG) and gene ontology (GO) databases. The metabolic pathway networks of *T. rubrum* were partially reconstructed
by searching for known pathway homologs in the Kyoto Encyclopedia of Genes and Genomes database.

**cDNA Microarrays.** PCR fragments used for printing the microarray chip were amplified from the EST library in 96-well plates using vector-PCR amplification with T7 and SP6 universal primers. PCR products were analyzed on gels to confirm the success of the reactions and were subsequently purified using MultiScreen-PCR plates (Millipore). Purified PCR products were resuspended in 50 µl of 3× SSC (0.45 M sodium chloride, 0.045 M sodium citrate [pH 7.0]) to produce the microarrays. A set of microarrays containing a total of 11,232 spots (including 10,250 clones in the form of PCR products and 982 controls in each block, including blank, negative, and positive controls were spotted in duplicate on slides (Corning) with a Cartesian® arrayer. The spotted cDNA was cross-linked to the surface of the slides (at 65 mJ) using a StrataLinker instrument and washed with 1% SDS to minimize background signal. Slides were subsequently placed in a blocking solution containing 0.2 M succinic anhydride and 0.05 M sodium borate prepared in 1-methyl-2-pyrrolidinone for 20 min, washed for 2 min in 95°C water, and rinsed five times in 95% ethanol. Slides were spin-dried at 500 rpm for 5 min and stored for future hybridizations.

**MIC determinations.** The broth microdilution assay for antifungal susceptibility testing of dermatophytes was previously developed as a modification of the National Committee for Clinical Laboratory Standards (NCCLS) M38-A method (21). RPMI 1640 medium (GIBCO BRL, Barcelona, Spain) with L-glutamine but without sodium bicarbonate and buffered at pH
7.0 with 0.165 M morpholinepropanesulfonic acid (MOPS) (Sigma, Madrid, Spain) was used for broth microdilution susceptibility testing. Test concentrations for KTC and AMB ranged from 0.01 to 64.0 µg/ml. A standardized inoculum was prepared by counting the microconidia microscopically. Cultures was subcultured onto PDA and incubated at 28°C for 7 days to produce conidia. Sterile normal saline (0.85%) was added to the agar slant, and the cultures were gently swabbed with a cotton-tipped applicator to dislodge the conidia from the hyphal mat. The mixture of conidia and hyphae fragments was filtered with a Whatman’s filter model 40 (pore size 8 µm), which retains hyphae fragments and only allows passage of T. rubrum microconidia. The suspension was transferred to a sterile centrifuge tube, and the volume was adjusted to 5 ml with sterile normal saline. Microdilution plates were set up in accordance with the NCCLS M38-A reference method. The final inoculum size was adjusted to between 0.4 × 10^4 and 5.0 × 10^4 colony-forming units (CFU)/ml. The microdilution plates were incubated at 30°C and were assessed visually after 72 hours of incubation. For azole agents, the minimum inhibitory concentration (MIC) was defined as the lowest concentration at which growth was markedly inhibited (approximately 50% growth of the control). For AMB, the MIC was defined as the lowest concentration at which 100% growth was inhibited. MIC results were recorded in micrograms per milliliter. Quality control strains Candida parapsilosis ATCC 22019 and Candida krusei ATCC 6258 were used to validate the susceptibility testing results.

**Cell culture and drug exposure for microarray experiments.** The isolates were subcultured onto PDA plates at 28°C. Stock inoculum suspensions of each isolate were
prepared for each experiment from 7-day-old cultures grown on PDA. The mixtures of conidia and hyphal fragments were collected in distilled water. These stock suspensions were diluted in RPMI medium to obtain inoculum sizes of $1.0 \times 10^6$ CFU/ml. For a single microarray experiment, a total of six 100-ml cultures were prepared (three independent 100-ml cultures were grown for each drug). All drug exposures were performed on the same day using the same starting culture to minimize experimental variations, and final inoculum sizes of $1.0 \times 10^4$ CFU/ml were reached. The cultures were incubated at 30°C and 140 rpm to saturation (4 days). An antifungal drug was added to three cultures at a concentration equivalent to 0.5 times the MIC (0.25 µg/ml KTC, 0.5 µg/ml AMB) and incubated. Three control cultures were treated with an appropriate amount of DMSO. Twelve hours after drug addition, three control cultures and three drug-treated cultures were harvested by filtration through Whatman no. 1 filter paper, washed thoroughly with sterile water, and quickly frozen in liquid nitrogen until RNA preparation.

**RNA Preparation.** The frozen *T. rubrum* cells were ground into a powder with a mortar and pestle in liquid nitrogen to facilitate cell disruption. Total RNA was isolated using the Qiagen RNeasy® Plant Mini Kit (Qiagen, Inc., Valencia, CA) according to the manufacturer’s instructions. The RNA concentration and purity were determined spectrophotometrically by measuring absorbance at 230, 260, 280, and 320 nm. The purity and integrity of the RNA were confirmed by agarose gel electrophoresis. Three independent sets of RNA from control and three independent sets of RNA from drug-treated cells were used to prepare six independent cDNA sets for AMB and KTC respectively. An aliquot
poly(A)$^+$ mRNA was isolated with Oligotex mRNA Mini Kit (Qiagen).

**Microarray hybridization.** First-strand cDNA was synthesized using Superscript™ II RT (Life Technologies/Invitrogen; Carlsbad, CA). An aliquot poly(A)$^+$ mRNA from 20 µg of total RNA sample was added to oligo(dT) (18- to 21-mer). The reaction mixture was heated to 65°C for 5 min and quickly chilled on ice. 5× first strand buffer (50 mM Tris-HCl, pH 8.3 at room temperature; 75 mM KCl; and 3 mM MgCl$_2$); 2× 0.1 M DTT; 10 mM each dATP, dCTP, dGTP, and TTP; and RNase OUT™ (40 U) were added to the mixture and incubated at 42°C for 2 min. 400 U of SuperScript™ II RT were added to the mixture and incubated at 42°C for an hour. To inactivate the reaction, the mixture was heated at 70 °C for 15 min.

Second strand cDNA was synthesized as follows: 5× second strand buffer (20 mM Tris-HCl, pH 6.9; 90 mM KCl; 4.6 mM MgCl$_2$; 0.15 mM $\beta$-NAD$^+$; 10 mM (NH$_4$)$_2$SO$_4$; 0.2 mM dNTP mix; *E. coli* DNA ligase (10 U); *E. coli* DNA polymerase I (40 U); and *E. coli* RNase H (2 U) were added to first strand reaction tube, and incubated for 2 hour at 16°C. To stop the reaction, 10 µl 0.5 M EDTA was added. Double-strand (ds) cDNA was purified using QIAquick columns (QIAGEN, Valencia, CA) and following the manufacturer’s instructions.

ds cDNA were then fluorescently labeled using BioPrime® DNA Labeling System (Life Technologies/Invitrogen; Carlsbad, CA). Those representing RNA from drug-treated cells was labeled using Cy5 and those representing RNA from control cells was labeled using Cy3.
ds cDNA was added to 2.5× random primers solution (50 mM Tris-HCl, pH6.8; 5 mM MgCl$_2$; 10 mM 2-mercaptoethanol; 300 µg/ml oligodeoxyribonucleotide primer), boiled at 100 °C for 5 min and then chilled on ice. 10× dNTP Mix for DNA labelling (0.12 mM dATP, dGTP,
dTTP; 0.06 mM dCTP; 1 mM Tris-HCl, pH 8.0; 0.1 mM EDTA); Cy3 (or Cy5) dCTP (0.06 mM); Klenow fragment (40 U) were added. The mixture was briefly centrifugated and was incubated at 37°C overnight away from light. 5μl 0.5 M EDTA (pH 8.0) was used to stop reaction. Labeled cDNA was purified using QIAquick columns and mixed with 2μg polyA; 5× Denharts; 3× SSC; yeast tRNA 24 μg; 25 mM HEPES, pH 7.0; 0.25% SDS, the mixture was heated at 100 °C for 2 min and cooled to room temperature and applied to the array slides under glass coverslips. Hybridization was performed at 65°C overnight in a Micro hybridization incubator (Robbins Scientific; Sunnyvale, CA). Slides were washed in 2× SSC (0.1%SDS), 1× SSC and 0.2× SSC sequentially, then were scanned at 5-μm resolution on a GenePix 4000B scanner (Axon Instruments, Inc). The Cy5- and Cy3-labeled DNA samples were scanned at 635 and 532 nm, respectively.

**Data analysis.** GenePix 6.0 software (Axon Instruments, Inc.) was used for image analysis and data visualization. Prior to data analysis, signals were normalized using a locally weighted scatterplot smoothing regression (LOWESS) algorithm (33) in the MIDAS software package (http://www.tigr.org/software/tm4) (25), with the smoothing parameter set to 0.33. Genes were considered to be differentially expressed if (i) average expression changed by at least 3-fold in three independent experiments performed with triplicate RNA samples or (ii) the change in gene expression was in the same direction (“increased” or “decreased”) in three experiments.

**Quantitative real-time RT-PCR.** Quantitative real-time reverse transcription (RT)-PCR was used to verify the microarray result. Aliquots of the RNA preparations from
untreated and treated samples used in the microarray experiments were saved for quantitative real-time RT-PCR follow-up studies. First-strand cDNAs were synthesized from 2 µg of total RNA in a 100-µl reaction volume using the SuperScript First-Strand Synthesis System for RT-PCR (Invitrogen, Carlsbad, CA) in accordance with the manufacturer’s instructions. Quantitative real-time PCR experiments were performed in triplicate using the 7000 Sequence Detection System (Applied Biosystems, Foster City, CA). Independent PCRs were performed using the same cDNA for both the gene of interest and the 18S rRNA, using the SYBR Green PCR Master Mix (Applied Biosystems). Gene-specific primers were designed for the genes of interest and the 18S rRNA using Primer Express software (Applied Biosystems) and are shown in Table I. The PCR cycle consisted of AmpliTaq Gold activation at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 15 s and annealing/extension at 60°C for 1 min. A dissociation curve was generated at the end of each PCR cycle to verify that a single product was amplified using software provided with the 7000 Sequence Detection System. The changes in fluorescence of SYBR Green I dye in each cycle were monitored by the system software, and the threshold cycle (C_\text{T}) above the background for each reaction was calculated. The C_\text{T} value of 18S rRNA was subtracted from that of the gene of interest to obtain a ΔC_\text{T} value. The ΔC_\text{T} value of an arbitrary calibrator (e.g., untreated sample) was subtracted from the ΔC_\text{T} value of each sample to obtain a ΔΔC_\text{T} value. The gene expression level relative to the calibrator was expressed as 2^{-ΔΔC_\text{T}}.

RESULTS
**MIC Determinations.** The susceptibilities of *T. rubrum* to antifungal compounds were determined. The antifungal drugs used were KTC and AMB. Based on those experiments mentioned above, the MIC values for the two drugs were determined as 0.5 µg/ml for KTC and 1 µg/ml for AMB.

**Construction of cDNA Libraries.** A total of 3,816 contigs and 7,269 singlets were found, which constitute the 11,085 *T. rubrum* assembled EST database. The ESTs were annotated using the databases mentioned above. It is noteworthy that some important genes involved in the growth, metabolism, signal transduction, pathogenesis and drug resistance of *T. rubrum* were identified, which could be used to determine important metabolic pathways in *T. rubrum*.

**Global gene expression results.** In total, 387 genes were differentially expressed upon exposure to KTC; 200 of which showed a significant increase in expression and 187 of which showed a significant decrease in expression. The data sets have been exported to the GEO (GEO accession number GSE 5014). The distribution of KTC-responsive genes and their biological roles are shown in Table II. Of the genes that showed a response to KTC, most were classed as “unknown function” (48.6%), and the others were classified as involved in amino acid transport and metabolism (6.5%), lipid, fatty acid and sterol metabolism (4.4%), post-translational modification, protein turnover, chaperones (4.4%), and carbohydrate transport and metabolism (3.9%). Some genes among the 387 genes differentially expressed upon exposure to KTC were listed in Table III.
In total, 686 genes were differentially expressed upon exposure to AMB; 244 of which showed a significant increase in expression and 442 of which showed a significant decrease in expression. The distribution of AMB-responsive genes and their biological roles are shown in Table IV. Of the genes that showed a response to AMB, most were of unknown function (44.2%), and the others were classified as involved in amino acid transport and metabolism (7.7%), translation, ribosomal structure and biogenesis (4.8%), lipid, fatty acid, and sterol metabolism (3.5%), and inorganic ion transport and metabolism (2.5%). Some genes among the 686 genes differentially expressed upon exposure to AMB were listed in Table V.

**Validation of microarray data by real-time RT-PCR.** Real-time quantitative RT-PCR was conducted to validate microarray data using the same RNA from the original microarray experiment. Ten genes (5 per drug) were tested not only to confirm their roles in the response of *T. rubrum* to the respective drugs (e.g. *ERG11*, *ERG6* and *ERG26* for KTC; *GIT1*, *FKSI*, and *GASI* for AMB), but also to verify the novel responses identified in the present study (e.g. *ABCI* and *phiA* for KTC; *DW701717* and *DW708548* for AMB). The result of real-time PCR analysis was shown in Fig.1. There was a strong positive correlation (r = 0.92) between the two techniques, 6 genes showed upregulation and 4 genes showed downregulation in response to drug treatment, which confirmed the reliability of the microarray data.

**DISCUSSION**

**Gene expression responses to KTC.** The number and characteristics of the responsive
genes are shown according to biological function in supplement table 1. In our large data pool, most responsive genes are classed as being of “unknown function” (188 genes), indicating that the EST has not previously been identified and there is no amino-acid sequence homology with other proteins of known function.

In our experiment, some genes of the ergosterol biosynthesis pathway were upregulated in response to KTC, including genes encoding \textit{ERG11}, \textit{ERG26}, \textit{ERG3}, \textit{ERG6}, \textit{ERG4}, \textit{ERG24}, and \textit{ERG25}. \textit{ERG11} encodes theazole target enzyme lanosterol demethylase, whereas \textit{ERG26}, \textit{ERG3}, \textit{ERG6}, \textit{ERG24}, \textit{ERG4}, and \textit{ERG25} are functional downstream of \textit{ERG11}, which indicates that their induction is in response to ergosterol depletion. Changes in the transcription levels of genes of the ergosterol pathway are in agreement with previous studies showing that this pathway is the target of azoles (1, 17). The most highly differentially expressed gene in this category was \textit{ERG6} which showed a \textasciitilde37-fold induction in response to KTC. The \textit{ERG6} catalyzes a biosynthetic step not found in humans and disruption of this gene in \textit{Saccharomyces cerevisiae} has been shown to result in several compromised phenotypes, most markedly increased permeability. Inhibitors of the \textit{ERG6} gene product would make the cell increasingly susceptible to antifungal agents that would normally be excluded from the fungus and would allow for clinical treatment at lower dosages (14). We note that \textit{ERG6} may be a potential drug target of antifungal agents.

In addition to the ergosterol biosynthesis genes, transcription of seven additional genes involved in lipid metabolism was also increased, including \textit{CPO}, \textit{INO1}, \textit{CPR}, \textit{ATG15}, \textit{DW696584}, \textit{DW700960}, and \textit{DW406350}. \textit{CPR} (\textit{P450R}) is required for microsomal
eukaryotic cytochrome P-450 (CYP) monooxygenase activity, transferring both or sometimes just the first electron required for these reactions (38). The CYP enzymes are involved in the metabolism of foreign compounds such as lipophilic pollutants, pesticides, and drugs, as well as in many biosynthetic reactions, such as steroid, alkaloid, and terpenoid biosynthesis. Overexpression of CYP51 and FUS has been shown to produce different levels of KTC resistance in wild-type cells, indicating that the availability of CPR may limit the potential of overproduction of CYP51 as a mechanism of resistance to azole antifungal agents (37). Three genes involved in lipid metabolism were repressed in this study including *IVD*, *DW678488*, and *ECH*.

In addition, KTC induced the transcription of the multidrug resistance gene *ABC1*. The observation that KTC induced transcription of *ABC1* in *T. rubrum* is a key finding of this study. *ABC1*-encoded protein is similar to yeast ABC transporters (Pdr5 and Cdr1) believed to be involved in multidrug resistance, and *ABC1* gene transcripts are inducible by toxic drugs. It has previously been shown that treatment of wild-type *Magnaporthe grisea* cultures with the antifungal compounds miconazole and metconazole markedly increase *ABC1* transcript levels. An alternative hypothesis is that Abc1 provides a defense function during early stages of pathogenesis by acting as an efflux pump to provide resistance to antimicrobial compounds (32).

Our results showed that expression of PhiA was repressed by a factor of 5.3. It has been reported that cell-wall protein encoded by the *phiA* gene of *Aspergillus nidulans* has a critical role in the development of normal phialides, with *phiA*-deficient mutants displaying abnormal
phialides and marked reductions in conidiation. Melin et al. propose a more challenging hypothesis in which the fungus might sense an attack by toxic metabolites, and that synthesis of PhiA is initiated to produce conidia and ensure survival (19).

We found that transcription of the genes encoding eight ribosomal proteins (L35A/L37, L36, L6, L3, yl16a, 15.5kD/SNU13, S18 and S10) was downregulated significantly. Small reductions in the expression of ribosomal protein genes allow energy to be redistributed to allow increased expression of genes involved in protective responses, while maintaining a basal level of protein synthesis (34). It should be noted that not all ribosomal protein transcripts are repressed. Ribosomal protein S15/S22 and mitochondrial/chloroplast ribosomal protein L15/L10 were upregulated in our study.

We noted that DCW1, which encodes a putative mannosidase, was repressed significantly upon exposure to KTC. DCW1 is required for cell-wall biosynthesis during bud formation and is homologous to Dfg5p. The results of both homozygote-triplication tests and conditional expression strategies indicate that dfg5 and dcw1 mutations are synthetically lethal (28).

In total, our results showed that the azole antifungal inhibited cytochrome P450-dependent enzymes that were involved in the biosynthesis of cell-membrane sterols.

**Gene Expression Responses to AMB.** The number and characteristics of the responsive genes grouped according to biological function are shown in supplement table 2. As with KTC, most responsive genes (303 genes) were of “unknown function”.

Of the characterized genes, most were classified as involved in transport. Transcription
of genes associated with membrane transport was upregulated in this study, including genes encoding GIT1, DW706156, DW684040, DMT, DW406136, CCH1, and DW710650. But, some genes that encode proteins involved in membrane transport were downregulated in response to AMB, including genes encoding PTR2, GAP1, DW700599, DW684458, Atp1a1, NMT1 DW699236, DW686710, DW696869, MEAA, ATP2C1, DW707140, and CTR1. The polyenes are thought to intercalate into membranes, forming a channel through which cellular components, especially potassium ions, leak, and thereby destroying the proton gradient within the membrane (35). It is possible that damage to the cell wall would cause defects in the plasma membrane and affect transport of small molecules through the membrane.

In our experiments, it was found that AMB reduced the transcription of some genes that encode ribosomal proteins, such as L19, L28, L11, DW679066, L13, L4, S18, HS6-type (S12/L30/L7a), and YmL35. But AMB increased the expression level of the genes that encode L3, L34, and S27. Similar results have previously been obtained by Zhang et al (40).

It was found that AMB induces genes that encode stress-response-related proteins in T. rubrum, including HSP70, HSP104, EB801455, and UBI4; oxidative-stress proteins such as GSS, and AOX; ethanol-stress proteins such as TDH1. At the same time, AMB repressed the transcription of some stress-response-related genes, including HSP10, DW678423, and GPX; osmotic-stress proteins HOR2.

In the present study, the transcription of several genes involved in lipid, fatty acid, and sterol metabolism was increased, such as DW696584, EB801458, DW682185, IVD, DW694010, DW688343, DW684992, and some genes were repressed, including DW703105,
The polyenes are a class of antifungal drugs that target cell membranes containing ergosterol. The specificity of the drugs for ergosterol-containing membranes is thought to be due to an interaction between AMB and ergosterol in the membrane, although the mechanism of this interaction is unknown. Downregulation of certain ergosterol biosynthesis genes in response to AMB may be indicative of an attempt by the organism to use alternate sterols or sterol intermediates in the cell membrane (17).

The transcription of some genes involved in maintenance of cell-wall integrity and signal transduction pathways was also repressed, including FKS1, SUN4, DW699324, GAS1, DW681613, SPS1, DW703091, STE7, DW703091, and DW695308. However, transcription of DW679073 was increased. In yeast, FKS1 encodes a subunit of the yeast 1,3-β-glucan synthase (18) and GAS1 is involved in remodelling of 1,3-β-glucan (23), loss of either of these two plasma-membrane-localized proteins results in significantly reduced levels of 1,3-β-glucan in the wall and in the formation of viable but swollen cells (23, 24).

Several multidrug resistance genes were affected in our experiments. Transcription of DW690470 and DW708548 was increased, whereas transcription of the gene DW698195 and DW701717 was reduced.

Responses in genes involved in regulation of cellular transport is in agreement with the mechanism of action of AMB, which binds membrane ergosterol to form pores that disrupt the membrane and cause leakage of ions and small molecules from the cell (12). The leakage
of ions and nutrients owing to formation of pores in the membrane is partly compensated by increased expression of transmembrane transporters (40). The regulation of oxidative-stress-response genes with AMB is consistent with the proposed oxidative damage caused by this antifungal agent (33, 34). The changes of transcription of some genes involved in maintenance of cell-wall integrity and signal transduction pathways are in agreement with previous observations that cell-wall components can affect interactions between AMB and the cytoplasmic membrane (11). The data presented here indicate that membrane reconstruction, cell stress, and improvement of cell-wall integrity are the main responses of T. rubrum to AMB.

In conclusion, our knowledge of the molecular biology of T. rubrum is still in its infancy, and it is difficult to elucidate the entire biology of T. rubrum. The conclusions drawn in this study are only preliminary and more genes will be identified and annotated. The T. rubrum microarray studies described here have revealed the gene-expression changes with two classes of antifungal agents to be consistent with their known mechanisms of action. There were also some specific findings in T. rubrum that differ from previous reports, this may be the main interest of the T. rubrum microarray studies. The study lays the groundwork for antifungal drug development using microarray studies to identify gene expression profiles.

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**FOOTNOTES**

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Fig.1. The relative fold change for 10 genes listed in Table I determined by quantitative real-time RT-PCR.

Table I Gene-specific primers used for real-time RT-PCR assays
<table>
<thead>
<tr>
<th>Target</th>
<th>Sequence&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Amplicon size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18S</td>
<td>F,5'- CGCTGGCTTCTTCTTAGAGGACTAT -3'</td>
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<tr>
<td></td>
<td>R,5'- TGCCTCAAAACTTCCATCGACTT -3'</td>
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<td></td>
<td>F,5'- TCAATTTCTGCGCTTTTGGTAT -3'</td>
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<td>ERG6</td>
<td>R,5'- CGCAAGATAGTGTTCGTCTTG -3'</td>
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<td>F,5'- CACTTCCTGGCCCTGTAGAGATC -3'</td>
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<td>ERG11</td>
<td>R,5'- GGAGTTTTCAATGTCAAGCAAGGTT -3'</td>
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<td>F,5'- TCCCGCTGTCTGACATTATA -3'</td>
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<td>R,5'- TGAAACCGTGTCGTAGATGGC -3'</td>
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<td>F,5'- CCAGCCTCCTCAAGGCTCTTT -3'</td>
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<td>ABC1</td>
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<td>F,5'- GCTGTCTGTGAAGTCAAGCCTGG -3'</td>
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<td>phiA</td>
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<td></td>
<td>F,5'- TCGGCTACATAGCAGGACACT -3'</td>
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<td>GIT1</td>
<td>R,5'- GCAGCGGAATACGGATCAGGATAATA -3'</td>
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<td>F,5'- TGCTGAGTTCGAGGAATGACC -3'</td>
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<td>FKS1</td>
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<td></td>
<td>F,5'- CGCTGCTGATGTCATGATA -3'</td>
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<tr>
<td>GAS1</td>
<td>R,5'- CAGGTAACGTAAGGCTCCAGGTATGTC -3'</td>
<td>51</td>
</tr>
</tbody>
</table>
Table II  KTC-responsive genes grouped by functional classification.

Translation, ribosomal structure and biogenesis

\[ \text{DW700253, DW693737, DHH1, DW701896, DW406697, DW698669, DW700103,} \]
\[ \text{DW702114, DW688959, EB801718, DW683699} \]

Transcription

\[ \text{ARO8, DHH1, SPS1, DW696767, DW701896, DW692217, DW699444, DW705142,} \]
\[ \text{DW704069, HIP1} \]

Signal transduction mechanisms

\[ \text{DW710420, SPS1, DW707020, DW406202, DW701611, DW692217, RBG2, DW701993,} \]
\[ \text{DW705142, DW684792, HIP1, DW696808} \]

Secondary metabolites biosynthesis, transport and catabolism

\[ \text{EB801566, DW703471, DW679333, DW692145, DW696127, DW702612, TMT1,} \]
\[ \text{DW695862} \]

RNA processing and modification

---

\( ^a \) F, forward; R, reverse.
Posttranslational modification, protein turnover, chaperones

GST, DW699217, DW702672, DW693794, DW678523, DW679113, DW679068, DW697385, QRI7, CLP, HMT1, DW682878, DW680862, DW710922, DW680089, DW709783, DW701227

Nucleotide transport and metabolism

DW698280, DW704384, DW690402

Nuclear structure

DW679616, CRM1/MSN5

Lipid metabolism

ERG6, CPO, ERG25, ERG26, ERG24, ERG4, CPR(P450R), DW406350, INO1, ATG15, ERG3, ERG11, DW696584, DW700960, IVD, DW678488, ECH

Intracellular trafficking, secretion, and vesicular transport

VPS33/slp1, ATG15, DW696952, DW702677, DW692357, CRM1/MSN5, YKT6, DW685279

Inorganic ion transport and metabolism

DW706483, SIT1, DW684278, DW707140, SUL1, DW701993, DW683256, DW684457, DW683754, DW705131

Extracellular structures

DW681163
Energy production and conversion

    DW698110, DW678636, DW698333, NDH, DW703655, DW691155, IDH, ACO2, 
    DW406745, DW703259, DW703411, DW697175, COX4, DW678789

DNA replication, recombination and repair

    DHH1, SPS1, DW406049, DW701896, DW692217, DW701869, HIP1

Defense mechanisms

    DW709093, DW405954

Cytoskeleton

    DW406457, SLA2, CAP2

Coenzyme metabolism

    DW701101, DW703971, DW684388, DW406323, DW406862, DW703639, DW697919, 
    DW697729, DW710087, PDX1, DW705554

Chromatin structure and dynamics

    NCA2, DW689402

Cell envelope biogenesis, outer membrane

    DW680921, DW699411

Cell division and chromosome partitioning

    NUF2

Carbohydrate transport and metabolism

    DW704437, SKN1, DW702573, DW680921, DW698094, SIT1, DW699411, DW707140,
Amino acid transport and metabolism

ERG26, ARO8, DW708866, DW678965, SIT1, DW406862, DW703560, DW707140, DW697919, IDH, DW699207, DW697889, DW697729, ILV6, SAT, GGT, DW707501, DW683263, DW703967, DW684457, DW705554, GAP1, DW705131, DW678242, DW696663

Other function

ABC1, TVP18, DW682938, DW683010, ANK, DW679066, DW691457, DW707096, RCI, DW678846, PhiA, DCW1, DW683510, DW694309, DW693546

Unknowns (data not shown)

---

Table III  Some genes differentially expressed upon exposure to KTC.

<table>
<thead>
<tr>
<th>Genebank accession</th>
<th>Gene symbol</th>
<th>Annotated functions</th>
<th>Fold induction/repression</th>
</tr>
</thead>
<tbody>
<tr>
<td>DW693552</td>
<td>ERG11</td>
<td>Lanosterol 14-alpha-demethylase</td>
<td>+3.6</td>
</tr>
<tr>
<td>DW705150</td>
<td>ERG26</td>
<td>C-3 sterol dehydrogenase</td>
<td>+11.7</td>
</tr>
<tr>
<td>DW695095</td>
<td>ERG3</td>
<td>Sterol C-5 desaturase</td>
<td>+3.6</td>
</tr>
</tbody>
</table>
EB801453  ERG6  Sterol C-24 methyltransferase  +37.5
DW692574  ERG4  Sterol C-24 reductase  +6.5
EB801509  ERG24  Sterol c-14 reductase  +8.6
EB801697  ERG25  Sterol C-4 methyloxidase  +11.9

Lipid metabolism
EB801711  CPO  Chloride peroxidase  +25.0
DW692676  INO1  Myoinositol-1-phosphate synthase  +3.8
DW679389  CPR  NADPH-cytochrome P450 reductase  +5.5

Lipase required for intravacuolar lysis of autophagic bodies  +3.7

DW685502  ATG15  Lipase required for intravacuolar lysis of autophagic bodies  +3.7
DW696584  Acyltransferase  +3.6
DW700960  Fatty acid desaturase  +3.6

Cytochrome P450 involved in gamma-hexachlorocyclohexane degradation  +4.0

DW684219  IVD  Isovaleryl-CoA dehydrogenase  -3.6
DW678488  3-oxoacid CoA transferase 1  -4.4
EB801635  ECH  Enoyl-CoA hydratase  -12.7

Cell-wall biosynthesis
DW680301  PhiA  Putative cell-wall protein  -5.3
DW687782  DCW1  Homologous to Dfg5p  -5.8
Multidrug resistance

\textit{DW680156} \textit{ABC1} \quad \text{Protein similar to yeast ABC transporters} \quad +6.3

Translation, ribosomal structure and biogenesis

\begin{tabular}{lll}
\textit{DW406697} & 60s ribosomal protein L35A/L37 & -3.1 \\
\textit{DW697947} & 60s ribosomal protein L36 & -3.9 \\
\textit{DW702114} & 60s ribosomal protein L6 & -3.4 \\
\textit{EB801718} & 60s ribosomal protein L3 & -3.4 \\
\textit{DW679066} & 60s ribosomal protein yl16a & -3.1 \\
\textit{DW683699} & 60s ribosomal protein 15.5KD/SUN13 & -8.3 \\
\textit{DW688959} & 40s ribosomal protein S18 & -3.4 \\
\textit{DW698669} & 40s ribosomal protein S10 & -3.2 \\
\textit{DW700253} & 40s ribosomal protein S15/S22 & +5.0 \\
\textit{DW693737} & Mitochondrial/chloroplast ribosomal protein L15/L10 & +4.8 \\
\end{tabular}

\(^a\) +, induction; -, repression.

\begin{table}[h]
\centering
\caption{AMB-responsive genes grouped by functional classification}
\end{table}
Translation, ribosomal structure and biogenesis

EB801718, EB801470, EB801473, DW685311, PSO2, DW680356,

DW707594, DW683559, DW405942, DW692644, DW710806, DW703493, DW680195,

DW680751, EIF2S1, DW698476, DW679709, RPL28, MAP, DW680448, DW679628,

DW696238, DW680472, DW406955, DW707974, DW692229, DW407252, DW680363,

DW692688, DW703685, DW405803, DW701284

Transcription

DW680640, DW701246, DW710589, DW709075, DW703421, DW705142, DW682224,

Ste7, DW696767, DW710806, DW680136, SPS1, DW406965, DW710354, DW696518,

DW703091, DW703443, DW707944, DW695755, DW706741, DW707823, SAT

Signal transduction mechanisms

CCH1, DW693741, DW704594, DW709075, DW689175, DW679073, DW682511, IQG1,

DW705142, DW686106, DW689727, DW706587, Ste7, DW680136, DW699564, SPS1,

DW711088, DW703091, DW703443, DW695308, ERG4_ERG24

Secondary metabolites biosynthesis, transport and catabolism

DW679333, DW694761, GSS, DW406408, DW697808, DW698800, DW406994, ACS,

DW710247, DW699753, DW694850, DW704981, CYP4/CYP19/CYP26, DW703137,

DW703105, FAH

RNA processing and modification

DW704990, DW686283, DW706110, DW688031, DW704126, DW699283, RCL1,
Posttranslational modification, protein turnover, chaperones

ECM4, DW680862, DW678739, DW683535, DW692280, DW683911, GST, UBI4, DW701186, DW682291, DW700821, EIF3S3, DW704126, DW685786, IMP1, PIN1, DW699217, DW695702, HSP10, SMT3, DW680298, DW706722, GPX, TEF4, NOB1

Nucleotide transport and metabolism

DW711065, DW700510, DW689577, DW678317, DW678423, ADE1, FUI1, DW688202, DW407054, DW405635

Nuclear structure

DW696987, DW679616, DW407252

Lipid, fatty acid, and sterol metabolism

DW696584, EB801458, DW682185, IVD, DW694010, DW684992, DW688343, MVD1, ELO2, DW690970, DW703202, IDI1, DW683501, DW678450, DW710649, DW703927, ACS, DW708678, EB801635, DW705750, DW699008, ERG4, DW697546, DW703105

Intracellular trafficking, secretion, and vesicular transport

DW678739, DW684040, RAB11, DW707807, DW710761, YOP1, DW696987, DW407047, DW693828, DW678450, IMP1, TOM20, DW690897, DW704279, DW407252, DW687382, DW706741, DW700202

Inorganic ion transport and metabolism

CCH1, Git1, DW710650, DW406136, DW698686, Atp1a1, DW699236, DW686710,
Extracellular structures

DW706156

Energy production and conversion

DW680874, DW703906, DW697175, DW679736, DW683295, NR1, COX1, DW697834, DW699315, DW691702, DW707870, DW704015, DW690897, DW687259, DW679360, DW708740, DW710675, DW702593, DW678789

DNA replication, recombination and repair

DW701121, MLH1, DW692105, DW682268, DW682224, PSO2, ESCO2, STE7, DW702799, DW678538, DW680136, SPS1, RFC3, DW703093, DW701832, RIM

Defense mechanisms

DW690470, DW698878, DW709093, DW698195, DW701717, PBP5

Cytoskeleton

DW696767, DW698466, DW711088

Coenzyme metabolism

DW679736, DW694761, DW685005, DW685690, DW700510, DW686283, DW710275, DW687999, DW706125, DW703166, DW407245, DW697919, DW680153, DW704497, DW705834, RFK, DW405632, DW680807, DW405667, FUI1, DW692131, DW706811, DW702415, PDX1

Chromatin structure and dynamics

SAS2, DW697867, DW704126
Cell motility and secretion

\textit{DW707795, DW707807, DW698818, DW706587, DW682876}

Cell envelope biogenesis, outer membrane

\textit{DW678251, DW678739, DW699411, DW686355, DW704907, DW698476, FKS1, DW703927, DW704279, DW702419, DW710254}

Cell division and chromosome partitioning

\textit{IQG1, DW688031, RFC3, DW711088, DW678876, DW703443}

Carbohydrate transport and metabolism

\textit{TDH1, GIT1, DW699411, DW696315, DW683911, DW708548, DW406136, DAK, DW708366, DW699236, DW706078, DW704437, DW698966, DW684458, DW702573, ALG3, DW700599, DW700064, DW700925, DW684254, DW707880, DW707140}

Amino acid transport and metabolism

\textit{NAD, Git1, DW707918, DW702180, DW706156, DW685847, DW684040, DW685690, DW708548, DW684721, DW684992, DW406136, DW706125, DW692131, DW704497, LYS1, DW407044, DW680807, DW406843, DW699236, DW698800, DW710247, DW702719, DW699268, DW680139, DW695702, DW696663, DW700424, DW697919, DW684458, DW707944, HPD, DW700599, DW700064, DW708740, DW709399, PTR2, NIT1, DW678242, DW680298, DW679625, DW686913, DW407054, DW697647, DW705341, DW702415, DW706684, GGT, GLY1, DW694726, DW707140, SAT, GAP1}

Other function
EB801455, AOX, EB801702, DW684745, DW687344, DW679066, DW681613, SED1,
DW707296, ATP17, DW699324, HOR2, GAS1, YAR1, DW698567, DW679576, SUN4

Unknowns (data not shown)

Table V  Some genes differentially expressed upon exposure to AMB.

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<th>Fold induction/ repression</th>
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<td>Sulfate ABC transporter ATP-binding protein</td>
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<td>DW708548</td>
<td>DMT</td>
<td>Permeases of the drug/metabolite transporter</td>
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<td>Synaptic vesicle transporter</td>
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<tr>
<td>DW679720</td>
<td>CCH1</td>
<td>Voltage-gated Ca2+ channels (alpha 1 subunits)</td>
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<tr>
<td>DW710650</td>
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<td>Outer membrane receptor proteins</td>
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<td>Dipeptide/tripeptide permease</td>
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<td>Accession</td>
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<td>Fold Change</td>
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<td>DW686710</td>
<td>Arsenite-translocating ATPase</td>
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<td>DW696869</td>
<td>Co/Zn/Cd efflux system component</td>
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<td>DW680730</td>
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<td>DW689541</td>
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Ribosomal structure and biogenesis

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<th>Description</th>
<th>Fold Change</th>
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<td>DW679628</td>
<td>60S ribosomal protein L19</td>
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<td>DW686754</td>
<td>60S ribosomal protein L28</td>
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<td>DW692688</td>
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<tr>
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<tr>
<td>DW680751</td>
<td>Ribosomal protein L13</td>
<td>-3.2</td>
</tr>
</tbody>
</table>
**Ribosomal protein S18**  <br> **DW692644**  -3.2

**Ribosomal protein HS6-type (S12/L30/L7a)**  <br> **DW703685**  -5.5

**Mitochondrial large ribosomal subunit YmL35**  <br> **DW698567**  -4.9

**60S ribosomal protein L3 and related proteins**  <br> **EB801718**  +24.5

**60s ribosomal protein L34**  <br> **EB801473**  +6.8

**40s ribosomal protein S27**  <br> **EB801470**  +11.5

### Stress response

**Probable chaperone protein DnaK**  <br> **DW701186**  +3.2

**Chaperone HSP104**  <br> **DW692280**

**Glutathione synthetase**  <br> **DW681149**  GSS  +4.2

**Alternative oxidase, mitochondrial precursor**  <br> **EB801466**  AOX  +38.3

**Putative stress response RCI peptide**  <br> **EB801455**  +56

**Glyceraldehyde-3-phosphate dehydrogenase**  <br> **EB801465**  TDH1  +8.3

**Ubiquitin**  <br> **DW688240**  UBI4  +3.9

**Co-chaperonin GroES (HSP10)**  <br> **DW689028**  HSP10  -4.3

**Predicted nucleotide kinase/nuclear protein**  <br> **DW678423**  -3.1

**involved oxidative stress response**

**Glutathione peroxidase**  <br> **DW710246**  GPX  -5.4

**DL-glycerol-3-phosphatase**  <br> **DW694000**  HOR2  -4.0
Lipid, fatty acid and sterol Metabolism

<table>
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<th>Description</th>
<th>Change</th>
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<td>DW678364</td>
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<tr>
<td>DW696584</td>
<td>Glycerol 3-phosphate acyltransferase</td>
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<td>EB801458</td>
<td>Carnitine O-acyltransferase</td>
<td>+5.3</td>
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<tr>
<td>DW682185</td>
<td>Acyl carrier protein phosphodiesterase</td>
<td>+4.5</td>
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<tr>
<td>DW684219</td>
<td>Isovaleryl-CoA dehydrogenase</td>
<td>+3.7</td>
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<tr>
<td>DW694010</td>
<td>Peroxisomal D3,D2-enoyl-CoA isomerase</td>
<td>+3.2</td>
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<td>DW688343</td>
<td>Carboxylesterase type B</td>
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</tr>
<tr>
<td>DW684992</td>
<td>Allophanate hydrolase subunit 1</td>
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</tr>
<tr>
<td>DW703105</td>
<td>Related to short-chain alcohol dehydrogenases</td>
<td>-5.2</td>
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<tr>
<td>DW697546</td>
<td>Cytochrome P450 CYP4/CYP19/CYP26 subfamilies</td>
<td>-4.7</td>
</tr>
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<td>DW692574</td>
<td>Ergosterol biosynthesis ERG4/ERG24 family</td>
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<tr>
<td>DW699008</td>
<td>3-methylcrotonyl-CoA carboxylase</td>
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<td>DW705750</td>
<td>Phosphomannomutase 2</td>
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<td>EB801635</td>
<td>Enoyl-CoA hydratase</td>
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<tr>
<td>DW680143</td>
<td>Acyl-coenzyme A synthetases/AMP-(fatty) acid ligases</td>
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<tr>
<td>DW703927</td>
<td>Phosphoethanolamine cytidylyltransferase</td>
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</tr>
<tr>
<td>DW708678</td>
<td>Lysosomal &amp; prostatic acid phosphatases</td>
<td>-3.8</td>
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<tr>
<td>Gene ID</td>
<td>Description</td>
<td>Log2 Fold</td>
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<td>DW710649</td>
<td>Short-chain acyl-CoA dehydrogenase</td>
<td>-3.6</td>
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<td>DW678450</td>
<td>Phosphatidic acid-preferring phospholipase A1, contains DDHD domain</td>
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<td>DW683501</td>
<td>3-oxo-5a-steroid 4- dehydrogenase</td>
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<td>DW698659</td>
<td>Isopentenyldiphosphate isomerase</td>
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<td>DW707302</td>
<td>Enoyl-[acyl-carrier-protein] reductase</td>
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<td>DW690970</td>
<td>3-hydroxyacyl-CoA dehydrogenase</td>
<td>-3.1</td>
</tr>
<tr>
<td>DW691391</td>
<td>ELO2 Fatty acid elongase</td>
<td>-3.1</td>
</tr>
<tr>
<td>DW686379</td>
<td>MVD1 Mevalonate pyrophosphate decarboxylase</td>
<td>-3.1</td>
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<tr>
<td>Cell-wall biosynthesis</td>
<td></td>
<td>-3.5</td>
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<tr>
<td>DW687269</td>
<td>FKS1 Catalytic subunit of 1,3-beta-D-glucan synthase</td>
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<td>DW678357</td>
<td>SUN4 Cell wall protein related to glucanases</td>
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<td>DW699324</td>
<td>1,3-beta-glucan synthase catalytic subunit</td>
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<td>DW703981</td>
<td>GAS1 Beta-1,3-glucanosyltransferase</td>
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<td>DW681613</td>
<td>GPI anchored protein</td>
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<td>DW679073</td>
<td>Integral membrane protein, interacts with FtsH</td>
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<td>Signal transduction</td>
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<td>DW703091</td>
<td>Mitogen-activated protein kinase MpkA</td>
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<td>DW699167</td>
<td>STE7 Mitogen-activated protein kinase kinase</td>
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</table>
Two-component phosphorelay intermediate

**DW695308**
involved in MAP kinase cascade regulation

**DW694457 SPS1**
Serine/threonine protein kinase
-3.2

Multidrug resistance

**DW690470**
RND family efflux system component
+3.9

Permeases of the drug/metabolite transporter

**DW708548**
(DMT) superfamily
+3.4

ABC-type multidrug transport system, ATPase component

**DW698195**
component
-4.8

**DW701717**
Na+-driven multidrug efflux pump
-5.1

*a* +, induction; -, repression.