The Antimicrobial Peptide MAF-1A Acts on the Transcriptional Response of Candida parapsilosis

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Rong Cheng
Medical college, Guizhou University

Wei Li
Department of Cardiovascular Medicine, Affiliated Hospital of Guizhou Medical University

Klarke M. Sample
Guizhou Provincial People's Hospital

Qiang Xu
Guizhou Provincial People's Hospital

Lin Liu
Guizhou Provincial People's Hospital

Fuxun Yu
Guizhou Provincial People's Hospital

Yingjie Nie
Guizhou Provincial People's Hospital

Xiangyan Zhang
Guizhou Provincial People's Hospital

Zhenhua Luo  luo8300@sina.com
Guizhou Provincial Peoples Hospital

Corresponding Author

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Abstract

Background Candida parapsilosis is a major fungal pathogen that can cause sepsis in man. Novel antifungal agents are urgently required due to the threat of resistance to current therapeutic strategies. MAF-1A is a novel cationic antimicrobial peptide isolated from Musca domestica and is effective against a variety of Candida species. However, its antifungal mechanism is still unclear. Here, RNA-seq was used to identify differentially expressed genes (DEGs) in Candida parapsilosis after MAF-1A exposure. And then, we want to understand how the antimicrobial peptide MAF-1A work as an antifungal agent.

Results The early (6 hour) response included 1122 genes with increased expression and 1065 genes with decreased expression. The late (18 hour) response was associated with the increased expression of 101 genes and decreased expression of 151 genes. When treated with MAF-1A from six to 18 hours, 42 genes were no longer expressed at elevated levels, and 25 genes that had a decreased expression pattern were reversed and demonstrated an increased expression pattern. KEGG enrichment showed that the DEGs caused by MAF-1A mainly involved amino acid synthesis and metabolism, oxidative phosphorylation, sterol synthesis and apoptosis. Conclusion These results indicate that MAF-1A may have multiple downstream effects in Candida parapsilosis. MAF-1A may exert antifungal activity by interfering with Candida parapsilosis cell membrane integrity and the function of certain organelles.

Background

Patients with a suppressed immune system are at a high risk for hospital-acquired fungal infections. The Candida genus accounts for the majority of fungal infections in the hospital setting. Although Candida albicans (C. albicans) is the most common pathogen of Candida infection, its dominance has decreased as the numbers of invasive infections by non-
*albicans* Candida (NAC) species have risen[1]. During the last two decades, epidemiological studies of fungal infections around the world have found that NAC infection has surpassed *C. albicans* as the most prevalent causes of invasive Candida infection[2]. It is therefore necessary to derive effective treatment programs to prevent NAC infection.

Among NAC infections, Candida *parapsilosis* (*C. parapsilosis*) is particularly problematic because it can form biofilms on central venous catheters and other medically implanted devices[1, 3]. Additionally, patients in intensive care units who have undergone total parenteral nutrition administered can be highly susceptible to *C. parapsilosis* infection, this includes undernourished children and neonates with low-birth-weights. Recent epidemiological studies (in various geographical regions worldwide) have shown that *C. parapsilosis* has become the second or third most prevalent following *C. albicans*[4]. If patients with a suppressed immune response are exposed to *C. parapsilosis*, the rate of infection is high and its biological characteristics such as toxicity, immune regulation, and drug resistance are quite different from *C. albicans*[4]. These interspecies specificities may affect host identification and clearance, as well as antifungal drug efficacy.

Common Candida pathogens have varying degrees of developed resistance and some represent a serious threat to human health[5], which requires the development of novel antifungal drugs. Antimicrobial peptides (AMPs) are an important part of the innate immune response for a variety of organisms[6]. AMPs are relatively small, and most exhibit strong cationic and amphiphilic properties. AMPs are widely available and are diverse with respect to length (20-100 amino acids), sequence and structure. It is currently uncommon for microbial infections to be resistant to this particular mode of action and therefore AMPs are expected to become an emerging source for novel antifungal drugs[7-9]. The *Musca domestica* antifungal peptide-1 (MAF-1) is a novel
cationic AMP isolated from the instar larvae of the housefly and has an excellent antimicrobial effect[10]. We have previously cloned the full-length MAF-1 gene and derived the 26 amino acid MAF-1A peptide from the MAF-1 structural domain. Despite the established antifungal effect of MAF-1A, the antifungal mechanism is still unclear[11]. As alluded to earlier, AMPs are amphiphilic and their cationic domains are susceptible to electrostatic interactions on the surface of cells, while the hydrophobic domains interact with the lipids that comprise the membranes, causing the cell membrane disintegration and cell death[12]. Whilst the aforementioned membrane damage mechanism is the canonical mechanism through which AMPs act, other mechanisms have been shown to exist. AMPs can have specific subcellular targets, such as inhibition of DNA, RNA, protein and cell wall synthesis [13, 14].

In recent years, the development of high throughput sequencing technologies has facilitated research on both the mechanisms by which antibacterial drugs function and microbes are resistant to drugs. For example, Iracane et al [15] found that HAC1 (CPAR2_103720) was a key gene involved in endoplasmic reticulum stress in C. parapsilosis using RNA-seq technology. In our previous study, Wang et al.[16] found that MAF-1A may inhibit C. albicans by affecting the cell wall, plasma membrane, protein synthesis and energy metabolism. However, the key action by which C. albicans responds to MAF-1A was not fully defined. In this study, we aim to expand upon how MAF-1A acts on fungal species and whether there are differences between the responses of C. albicans and C. parapsilosis. RNA sequencing (RNA-Seq) was used to elucidate the mechanism of the antifungal molecule ‘MAF-1A’ and the changes in gene expression it promotes in early (6 hour) and late (18 hour) stage (these time points were chosen according to the results of time-kill curves) C. parapsilosis growth.

Results
2.1. Minimum Inhibitory Concentration (MIC) Assay and Time-kill Curve

The MIC of MAF-1A against *C. parapsilosis* was determined to be 0.6 mg/mL. The Time-kill Curve of MAF-1A at MIC demonstrated a gradual antifungal effect during the first eight hours of *C. parapsilosis* culture (Figure 1). The number of cells gradually decreased and formed a downward trend for the first eight hours of culture. However, after eight hours the curve steep end and increased at a trajectory similar to the control group ending with approximately $3 \times 10^8$ fewer colonies.

2.2. Transcriptional Stress Response of *C. parapsilosis* to MAF-1A

The RNA-seq results from *C. parapsilosis* with MAF-1A after six hours and 18 hours showed that there were a total of 5747 expressed genes. A total of 2439 significantly differentially expressed genes were detected at the 6 hour and 18 hour time points for MAF-1A treated *C. parapsilosis* compared to the control (Figure 2) Among these genes, 2187 genes were found at six hours, which represented 38.05% of the total detectable genes. Whereas, 252 genes were found to be differentially expressed after 18 hours and accounted for 4.38% of the detectable genes. After six hours and 18 hours of MAF-1A treatment, we found that 67 genes that were significantly differentially expressed with opposite trends between the two MAF-1A treated time points compared with the control group (reversed genes 1, RG1 and reversed genes 2, RG2).

2.3. Verification of Differentially Expressed Genes

20 genes were selected, including 10 genes with increased expression and 10 genes with decreased expression, which were evenly drawn from the six hour and 18 hour time points to validate the RNA-Seq results by quantitative reverse transcription-polymerase chain reaction (qRT-PCR) (Figure 3).

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2.4. The protein-protein interaction (PPI) Network Analysis
The DEGs in C. parapsilosis that were treated for six hours with MAF-1A were used to construct a PPI network based on the STRING database. The PPI network contains a total of 624 nodes and 6264 edges, with a degree filter of ≥10 (Figure 4). The connectivity degree (dg) of multiple gene nodes in the PPI network was higher for genes including: UBI1 (dg = 146), glt1 (dg = 82), CDC28 (dg = 54), CYS4 (dg = 50), CYT1 (dg = 45), RPC40 (dg = 42), ARX1 (dg = 42), DIM1 (dg = 41), YTM1 (dg = 41), RIP1 (dg = 41). Subsequently, enrichment analyses were performed for these genes (Supplementary Figure 1).

2.5. Enrichment Analysis of DEGs Altered by MAF-1A
To obtain insights into the molecular mechanism of the antifungal mechanism of MAF-1A on C. parapsilosis, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis was performed for both the differentially expressed genes at the 6 hour time point and the RG1 and RG2 gene groups.

2.5.1 DEGs enrichment analysis at six hours
Among the DEGs at the 6 hour time point, 1122 genes with increased expression were enriched in 85 KEGG pathways, 20 of which were significant with a q-value of <0.05. The most significantly pathways with increased expression in C. parapsilosis after MAF-1A treatment were: Oxidative phosphorylation, Peroxisome, Citrate cycle (TCA cycle), Carbon metabolism, Cell cycle-yellow, MAPK signaling pathway - yeast, Meiosis - yeast, Fatty acid degradation and Autophagy - other (Table 1).

The genes with decreased expression were significantly enriched in Steroid biosynthesis, Biosynthesis of amino acids, Cysteine and methionine metabolism, Biosynthesis of antibiotics, Ribosome, RNA polymerase, Biosynthesis of secondary metabolites, RNA transport, Ribosome biogenesis in eukaryotes, Lysine biosynthesis, 2-Oxocarboxylic acid
metabolism, Pyrimidine metabolism, Glycine, serine and threonine metabolism, Purine metabolism (Table 2).

2.5.2. RG1 and RG2 gene enrichment analysis

The 42 genes in RG1 were enriched in 17 KEGG pathways, of which Oxidative phosphorylation was significant. Additionally, The 25 genes in RG2 were enriched in 13 KEGG pathways, of which Arginine biosynthesis, Biosynthesis of antibiotics, Biosynthesis of amino acids and Biosynthesis of secondary metabolites were significantly enriched (Table 3).

Moreover, the genes in RG1 and RG2 were enriched in 290 GO terms, 8 of which were significant (Table 4). The genes in RG1 are mainly involved with energy production and redox processes, whereas the genes in RG2 are associated with the anabolic process of various organic acids in cells.

Discussion

In recent years, C. parapsilosis infection has risen to the second or third commonly detected species of the Candida genus. In addition to its high infection rate, its etiology differs markedly from that of C. albicans [17]. C. parapsilosis is resistant to the conventional antifungal drugs echinocandins, azoles, and amphotericin B [18-20].

Moreover, few new drugs against C. parapsilosis infection have been reported in the scientific literature, which is an area this study aims to enrich. Antimicrobial peptides are considered to a potential source of novel antimicrobial drugs because of the identification of peptides with excellent antimicrobial properties, such as those described by Patocka et al [9]. Studies have shown that antimicrobial peptides mainly cause cell lysis and death by destroying the cell membrane and causing the leakage of cell contents [21-24]. However, mechanistic studies of antimicrobial peptides have determined that membrane interactions are a part of the complex mechanism of action, but not all. Park et al. [25]
found that buforin II can prevent the microorganisms from entering host cells through the cell membrane and exerts its antibacterial effect interference with DNA and RNA functions. Lee et al. [26] found that antifungal β-peptides cause cell death by entering the cell and disrupting the nucleus and vacuole function. Studies by Chileveru et al. [27] showed that human alpha-defensin 5 enters the cytoplasm of E. coli and interferes with cell division to exert an antibacterial effect. In our previous study, Wang et al [16] found MAF-1A inhibited the C. albicans by affecting multiple targets including the cell wall, membrane and ribosome. In this study, we show that MAF-1A affects multiple targets in C. parapsilosis, with the predominant changes involving the cell membrane, intracellular mitochondria, ribosome, and nucleic acid synthesis. It is believed that antimicrobial peptides may have multiple modes of action, but the exact mechanism of action has not been determined for MAF-1A. Our study suggests that intracellular targets may be a key site of action for MAF-1A and the enrichment analysis of DEGs suggest that MAF-1A may exert an antibacterial activity through a variety of aspects.

**Membrane Destruction**

The genes with decreased expression after six hours were significantly enriched in the Steroid biosynthesis pathway (Figure 5) and a total of 14 genes were enriched (ERG1, ERG3, ERG6, ERG7, ERG9, ERG11, ERG25, ERG26, ERG27, ERG2, ERG4, ERG5, ERG24, SPBC16A3.12c. Azole antifungal agents exert an antifungal action by inhibiting the synthesis of Ergosterol, which is a major component of fungal cell membranes [28]. It has been found that overexpression of ERG11 (encoding lanosterol 14-demethylase) is one of the main causes of azole antifungal drug resistance. Members of the ERG gene family primarily encode proteins involved in the ergosterol biosynthesis pathway, of which lanosterol 14-demethylase is a key enzyme. In this study, MAF-1A affected the decreased expression of
14 genes related to sterol synthesis including the ERG11 gene, which suggests that MAF-1A interferes with the synthesis of ergosterol. We hypothesize that MAF-1A could also exert an antifungal effect by disrupting the normal function of the cell membrane. Additionally, 10 genes that had increased expression after six hours were significantly enriched in the fatty acid degradation pathway (Figure 6). This indicates that the composition of the cell membrane could also be affected by MAF-1A.

**Protein synthesis**

After six hours, there was a significant enrichment of genes that had lower expression in a number of pathways involved with amino acid biosynthesis, such as the biosynthesis of amino acids, Cysteine and methionine metabolism, and Ribosomes. The genes ARG3, ARG5, and ARG1 in RG2 are enriched in multiple protein synthesis related pathways. It is suggested that MAF-1A may interfere with the normal synthesis of proteins and affect the normal growth of *C. parapsilosis*. This prospect is also supported by Florin et al. [29], who found that the antibacterial peptide Api137 interferes with bacterial growth by trapping release factors on the ribosome, thus inhibiting translation. Moreover, Gagnon et al. [30] reported that the antimicrobial peptide Bac71-35 binds to the 70S ribosome of *Thermus thermophilus* and interferes with translation initiation. In the present study, the presence of MAF-1A resulted in the decreased expression of multiple genes involved with ribosomes and translational processes. Therefore, it is possible that MAF-1A may exert antifungal activity by interfering with the translation process, which would affect the normal growth of *C. parapsilosis*.

**Affect Mitochondrial Function**

The 42 genes with increased expression at the six hour time point were enriched in the oxidative phosphorylation pathway. These genes are mainly involved in the respiratory chain electron transport process on the mitochondrial inner membrane, such as the
process of NHD release of H+, ATP synthase (Figure 7). A total of six genes in RG1 genes were also enriched in this pathway (COX15, nuo-21, QCR2, QCR8, QCR7 and COR1). Glerum et al. [31] found that COX15 encodes an indispensable mitochondrial protein for *Saccharomyces cerevisiae* cytochrome oxidase. Cytochrome oxidase as a terminal enzyme in the respiratory electron transport chain, which essential for the synthesis of ATP. Reactive oxygen species (ROS) is mainly caused by oxidative phosphorylation of ATP to disorganize electron transport in mitochondria [32]. ROS such as hydrogen peroxide and hydroxyl radicals can cause serious damage to proteins, lipids, and nucleic acids, leading to irreversible damage or even the death of cells. Eukaryotes mainly prevent cell damage by inducing oxidative stress detoxification and repairing protein-related gene expression to prevent ROS accumulation. In our study, GO enrichment analysis of the RG1 gene group showed that a total of seven genes with increased expression were involved in the redox process (POX9, COX15, nuo-21, AAE001134, QCR7, RGI1 and namA). This process helps the cells remove accumulated ROS. Due to the increased expression of these genes under the action of MAF-1A, it is possible that MAF-1A could cause oxidative phosphorylation, which would disrupt electron transfer in mitochondria to produce ROS and cause damage to the cells.

*C. parapsilosis* has an unusual mitochondrial genome molecular architecture, which consists of linear DNA molecules of 30.9-Kbp long and terminating with specific telomeric structures on both sides (738-Kbp long). This differs from telomeres at the ends of eukaryotic nuclear chromosomes, especially humans [33]. MAF-1A appears to interfere with the expression of multiple genes related to the function and mitochondrial composition of *C. parapsilosis*, therefore it is possible that MAF-1A may interfere with the normal function of *C. parapsilosis* mitochondria.

**Induction of Apoptosis**
The genes with increased expression after six hours were enriched in the Cell cycle-yeast, MAPK signaling pathway-yellow, Meiosis-yeast, Autophagy-other (and others) pathways. This suggests that MAF- 1 may induce the up-regulation of apoptosis-related genes in C. parapsilosis cells, triggering the initiation of apoptosis and thereby exerting an antifungal effect. There is precedent for this mechanism, Lee et al. [34] found that resveratrol induced fungal apoptosis through a caspase-dependent mitochondrial pathway. Moreover, Lopes et al. proposed that peptide sequences from Osmotin-like proteins and thaumatin-like proteins (derived from plant defense systems) did not damage the yeast cell membrane and cell wall integrity, but rather induced ROS production and apoptosis [35].

**Conclusions**

In summation, it is possible that MAF-1A has multiple targets in C. parapsilosis (akin to, but possibly distinct from C. albicans). Most of the DEGs identified through the RNAseq analysis were related to intracellular structures, which as the mitochondria and ribosomes in C. parapsilosis. The RNAseq data has provided potential future directions for the study of the antifungal molecular mechanisms of MAF-1A and has highlighted potential pathways that could contribute to resistance to its actions.

**Methods**

5.1. Strains and growth conditions

In this study, transcriptional profiling was performed on the C. parapsilosis reference strain ATCC22019. The strain was preserved in goat blood and stored at -80°C. Subsequently C. parapsilosis was streaked on Sabouraud Dextrose Agar (SDA) plates (Sangon, Shanghai, China) at 35°C as described by Lis et al [36]. Experiments involving treatment with the MAF-1A were conducted using Sabouraud Dextrose Broth (SDB) (Sangon, Shanghai, China).
5.2. Peptide Synthesis

MAF-1A was synthesized by Sangon Biotech (Shanghai, China) as a linear peptide consisting of a 26 amino acid sequences as follows: KKFKETADKLIESAKQQLESLAKEMK. Analytical high-performance liquid chromatography (HPLC) was used to confirm that the purity was higher than 95%. The peptide was dissolved in sterile ultrapure water at 5 mg/mL and stored at -20°C.

5.3. MIC Determinations and Time-kill Curve

Antifungal assays were performed as per the directives of the Clinical and Laboratory Standards Institute (CLSI) M27-A3. Briefly, cultures were grown for 24 hours at 35°C and resuspended in SDB. The cellular concentration was adjusted to approximately 0.5x10^3-2.5x10^3 CFU/mL and 100 μl of the suspension was added to each well of a 96-well polypropylene microplate (NEST, Wuxi, China). MAF1A was added to a final concentration of 0.1 mg/mL to 1.2 mg/mL in triplicate. After incubation at 35°C for 24 hours, the A492nm value was measured by a Microplate Reader (BioTek Synergy H1, Vermont, USA). The MIC was defined as the lowest drug concentration showing 80% growth inhibition compared to the drug-free control. The following formulas were used:

and

The methodology for producing time kill curves was adapted from Li et al and Sun et al [37, 38]. The C. parapsilosis suspensions were mixed with MAF-1A (at the MIC concentration) in triplicate and cultured at 35°C. 100μl aliquots were removed from each test solution at predetermined time points (0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and 24 hours). After 100-fold dilutions, a 10 μl aliquot from each dilution was streaked in triplicate onto SDA plates for colony count determination after incubation at 35°C for 24 hours. Sterile ultrapure water was used as a control.
5.4. Total RNA Extraction

*C. parapsilosis* was inoculated into SDB medium (Sangon, Shanghai, China) and cultured at 35°C for 24 hours. *C. parapsilosis* was treated with MAF-1A at MIC for six hours (CPAS) and 18h (CPBS), before being harvested for RNA extraction. The untreated cultures were used as the control (six hours, CPAC; 18 hours, CPBC). Total RNA was extracted with RNAiso Plus (Takara, Dalian, China) according to the manufacturer’s instructions. The concentration and quality of the RNA samples was determined using a NanoDrop 2000 (Thermo Fisher Scientific, Wilmington, DE, USA) and an Agilent 2100 bioanalyzer (Agilent Technologies, CA, USA). Library preparation was conducted using NEBNext® UltraTM RNA Library Prep Kit (NEB, USA) as per the manufacturer’s instructions. The purified library was quantified using an Agilent 2100 bioanalyzer, and qRT-PCR was used to accurately quantify the effective concentration of the library. The library was prepared and subsequently sequenced using a Novoseq sequencer (Illumina, USA) by Novogene Corporation (Beijing, China), which produced 150 bp paired-end reads.

5.5. Differential expression analysis

The raw reads were filtered to obtain high-quality clean reads for subsequent analysis and mapped to the reference genome *C. parapsilosis* (assembly ASM18276v2) from the National Center for Biotechnology Information (NCBI), US. using HISAT2 [39] v2.0.5. Differential expression analysis between the two conditions was performed using the Bioconductor software package DESeq2 [40] in R (1.16.1), the estimated gene expression levels were reported using FPKM (Fragments Per Kilobase of transcript sequence per Millions of base pairs sequenced) [41] and compared to each other using log2 FC. The P value was adjusted to generate the false discovery rate (q value) using the method described by Benjamini-Yekutieli [42], which assigns the significance threshold for differentially expressed genes as q= < 0.05.
5.6. Network analysis of differentially expressed genes

To further understand the functions of the DEGs, we implemented Gene Ontology (GO) enrichment through the Bioconductor software clusterProfiler [43] (3.4.4) in R, which uses the KEGG database to identify enriched pathways. PPI analysis of differentially expressed genes was based on the STRING database [43], which provided comprehensive information about interactions between proteins and the network was constructed using Cytoscape [44] (3.6.1).

5.7. Validation of RNA-Seq by quantitative RT-PCR

To confirm the RNA-Seq results, 20 DEGs (10 with increased expression and 10 with decreased expression) were selected for qRT-PCR validation. The reaction was performed using a SYBR Premix Ex TaqTM Kit (Takara) according to the manufacturer’s protocol. The reaction procedure of 40 cycles of 95°C for 30 seconds and 95°C for 5 seconds and 60°C for 30 seconds was conducted using a BIO-RAD CFX-Connect Real-Time System. The gene expression level was determined using the $2^{-\Delta\Delta Ct}$ method [45] using the house-keeping gene 18S rRNA to normalized the candidate gene abundance. The primers used in this study are listed in Supplementary Table 1.

5.8. Statistical Analysis

Data in this study are shown as mean±standard deviation. Statistical calculations were using IBM SPSS Statistics 23.0. A p value and q Value less than 0.05 were significant.

Declarations

Ethics approval and consent to participate: Not applicable.

Consent for publication: Not applicable.

Availability of data and materials: The datasets used and analyzed during this study are available from the corresponding author on request.
Competing interests: The authors declare that they have no competing interests.

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Author Contributions: Zhen-hua Luo and Xiangyan Zhang conceived of the study; Zhen-hua Luo and Rong Cheng designed the experiments; Rong Cheng, Wei Li, Klarke M. Sample, Qiang Xu, Lin Liu, Fuxun Yu, Nie Y analyzed the data; Rong Cheng, Zhen-hua Luo and Klarke M. Sample performed the experiments; Rong Cheng, Zhen-hua Luo, Klarke M. Sample wrote the manuscript. All authors reviewed the results and approved the final version of the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

DEGs: Differentially expressed genes
C. albicans: Candida albicans
C. parapsilosis: Candida parapsilosis
NAC: Non- albicans Candida
AMPs: Antimicrobial peptides
MAF-1: Musca domestica antifungal peptide-1
MIC: Minimum Inhibitory Concentration
RG1: Reversed genes 1
RG2: Reversed genes 2
qRT-PCR: Quantitative reverse transcription-polymerase chain reaction
PPI: Protein-protein Interaction
GO: Gene Ontology
KEGG: Kyoto Encyclopedia of Genes and Genomes
SDA: Sabouraud Dextrose Agar
SDB: Sabouraud Dextrose Broth
HPLC: High-performance liquid chromatography
CLSI: Clinical and Laboratory Standards Institute
FPKM: Fragments Per Kilobase of transcript sequence per Millions of base pairs sequenced

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Tables

**Table 1.** Significantly enriched KEGG pathways for the genes with increased expression after six hours. A q value of <0.05 was set as the significance threshold.

| KEGGID       | Description                              | p Value       | q Value       |
|--------------|------------------------------------------|---------------|---------------|
| cd00100      | Steroid biosynthesis                     | 1.64×10⁻⁸     | 1.40×10⁻⁴     |
| cd01230      | Biosynthesis of amino acids              | 5.95×10⁻⁷     | 2.53×10⁻⁴     |
| cd00270      | Cysteine and methionine metabolism       | 4.80×10⁻⁶     | 1.36×10⁻⁴     |
| cd01130      | Biosynthesis of antibiotics              | 1.07×10⁻⁵     | 2.27×10⁻⁴     |
| cd03010      | Ribosome                                 | 3.01×10⁻⁵     | 5.12×10⁻⁴     |
| cd03020      | RNA polymerase                           | 2.14×10⁻⁴     | 3.03×10⁻¹     |
| cd01110      | Biosynthesis of secondary metabolites    | 3.68×10⁻⁴     | 4.47×10⁻¹     |
| cd03013      | RNA transport                            | 9.34×10⁻⁴     | 8.83×10⁻¹     |
| cd03008      | Ribosome biogenesis in eukaryotes        | 9.35×10⁻⁴     | 8.83×10⁻¹     |
| cd00300      | Lysine biosynthesis                      | 2.31×10⁻³     | 1.96×10⁻¹     |
| cd01210      | 2-Oxocarboxylic acid metabolism          | 2.66×10⁻³     | 2.05×10⁻¹     |
| cd00240      | Pyrimidine metabolism                    | 3.54×10⁻³     | 2.40×10⁻¹     |
| cd00260      | Glycine, serine and threonine metabolism | 3.67×10⁻³     | 2.40×10⁻¹     |
| cd00230      | Purine metabolism                        | 5.08×10⁻³     | 3.09×10⁻¹     |

**Table 2.** Significantly enriched KEGG pathways for the genes with decreased expression after six hours. A q value of <0.05 was set as the significance threshold.
| KEGG ID | Description                        | p Value   | q Value   |
|---------|------------------------------------|-----------|-----------|
| cdu00190 | Oxidative phosphorylation          | 3.34×10^{-12} | 2.84×10^{-10} |
| cdu04146 | Peroxisome                         | 2.02×10^{-8} | 8.56×10^{-7} |
| cdu00200 | Citrate cycle (TCA cycle)          | 2.21×10^{-5} | 6.27×10^{-4} |
| cdu01200 | Carbon metabolism                  | 3.50×10^{-5} | 7.44×10^{-4} |
| cdu04111 | Cell cycle - yeast                 | 9.20×10^{-5} | 1.56×10^{-3} |
| cdu04011 | MAPK signaling pathway - yeast     | 8.17×10^{-4} | 1.16×10^{-2} |
| cdu04113 | Meiosis - yeast                    | 2.35×10^{-3} | 2.80×10^{-2} |
| cdu0071  | Fatty acid degradation             | 2.63×10^{-3} | 2.80×10^{-2} |
| cdu04136 | Autophagy - other                  | 4.03×10^{-3} | 3.80×10^{-2} |

**Table 3.** Significantly enriched KEGG pathways for the genes in RG1 and RG2. A q value of <0.05 was set as the significance threshold.

| sort | KEGG ID | Description                                      | p Value   | q Value   |
|------|---------|--------------------------------------------------|-----------|-----------|
| RG1  | cdu00190| Oxidative phosphorylation                        | 1.34×10^{-5} | 2.27×10^{-4} |
|      | cdu00220| Arginine biosynthesis                             | 1.97×10^{-5} | 2.56×10^{-4} |
|      | cdu01130| Biosynthesis of antibiotics                        | 4.09×10^{-3} | 2.39×10^{-2} |
|      | cdu01230| Biosynthesis of amino acids                       | 5.52×10^{-3} | 2.39×10^{-2} |
|      | cdu01110| Biosynthesis of secondary metabolites             | 1.21×10^{-2} | 3.92×10^{-2} |
| RG2  | cdu00190| Oxidative phosphorylation                        | 1.34×10^{-5} | 2.27×10^{-4} |
|      | cdu00220| Arginine biosynthesis                             | 1.97×10^{-5} | 2.56×10^{-4} |
|      | cdu01130| Biosynthesis of antibiotics                        | 4.09×10^{-3} | 2.39×10^{-2} |
|      | cdu01230| Biosynthesis of amino acids                       | 5.52×10^{-3} | 2.39×10^{-2} |
|      | cdu01110| Biosynthesis of secondary metabolites             | 1.21×10^{-2} | 3.92×10^{-2} |

**Table 4.** The significant enriched GO terms of RG1 and RG2. A q value of <0.05 was set as the significance threshold for enrichment.

| sort | Category | GO ID    | Description                                      | p Value   |
|------|----------|----------|--------------------------------------------------|-----------|
| RG1  | BP       | GO:0006091| generation of precursor metabolites and energy    | 8.71×10^{-5} |
|      | BP       | GO:0055114| oxidation-reduction process                      | 8.49×10^{-4} |
|      | BP       | GO:0016053| organic acid biosynthetic process                 | 5.27×10^{-4} |
|      | BP       | GO:0046394| carboxylic acid biosynthetic process              | 5.27×10^{-4} |
|      | BP       | GO:0044283| small molecule biosynthetic process               | 1.56×10^{-3} |
|      | BP       | GO:0006082| organic acid metabolic process                    | 2.34×10^{-3} |
|      | BP       | GO:0019752| carboxylic acid metabolic process                 | 2.34×10^{-3} |
|      | BP       | GO:0043436| oxoacid metabolic process                        | 2.34×10^{-3} |
| RG2  | BP       | GO:0006091| generation of precursor metabolites and energy    | 8.71×10^{-5} |
|      | BP       | GO:0055114| oxidation-reduction process                      | 8.49×10^{-4} |
|      | BP       | GO:0016053| organic acid biosynthetic process                 | 5.27×10^{-4} |
|      | BP       | GO:0046394| carboxylic acid biosynthetic process              | 5.27×10^{-4} |
|      | BP       | GO:0044283| small molecule biosynthetic process               | 1.56×10^{-3} |
|      | BP       | GO:0006082| organic acid metabolic process                    | 2.34×10^{-3} |
|      | BP       | GO:0019752| carboxylic acid metabolic process                 | 2.34×10^{-3} |
|      | BP       | GO:0043436| oxoacid metabolic process                        | 2.34×10^{-3} |
Time-kill curves of MAF-1A under MIC concentration for C. parapsilosis. The mean growth of three C. parapsilosis cultures was recorded using the number of colony-forming units per milliliter to the logarithmic base of 10 (Log10 CFU/ml) every two hours for 24 hours.
Figure 2

Gene expression changes in C. parapsilosis following MAF-1A treatment. The volcano plots of the differentially expressed genes. (a) The volcano plot depicts the log2 FC (fold change) expression after six hours of treatment with MAF-1A, C. parapsilosis was treated with MAF-1A at MIC for six hours (CPAS) and 18h (CPBS), before being harvested for RNA extraction. The untreated cultures were used as the control (six hours, CPAC; 18 hours, CPBC). The expression of 1122 genes was significantly increased compared with 1065 genes that were significantly
decreased (q=<0.05). (b) The volcano plot depicts the log2 FC in expression after 18 hours of treatment with MAF-1A, the expression of 101 genes was significantly increased compared with 151 genes that were significantly decreased (q=<0.05).

(c) The gene expression Venn diagram revealed two gene groups which have opposite trends and were labeled RG1 and RG2; CPAS vs. CPAC_up: genes with increased expression after six hours; CPAS vs. CPAC_down: genes with decreased expression after six hours; CPBS vs. CPBC_up: genes with increased expression after 18 hours; CPBS vs. CPBC_down: genes with decreased expression after 18 hours.
Comparison of the expression levels of 20 genes between RNA-Seq and qRT-PCR.
(a) 6 hour time point. (b) 18 hour time point. RNAseq n=3 and qRT-PCR n=3. Error bars represented as the standard error of the mean (SEM).

PPI network of the DEGs resulting from MAF-1A treatment of C. parapsilosis for six hours. Node sizes are correlated with node importance; red nodes denote genes with increased expression and green nodes denote those genes with decreased expression.
Figure 5

Significantly enriched KEGG pathways in Steroid biosynthesis. The DEGs with decreased expression are marked in green. Permission for publication granted by the Kyoto Encyclopedia of Genes and Genomes, Kyoto University, Japan.
Figure 6

Significantly enriched KEGG pathways in Fatty acid degradation. The DEGs with increased expression are marked with a red outline. Permission for publication granted by the Kyoto Encyclopedia of Genes and Genomes, Kyoto University, Japan.
Figure 7

Significantly enriched KEGG pathways in Oxidative phosphorylation. The DEGs with increased expression are marked with a red outline. Permission for publication granted by the Kyoto Encyclopedia of Genes and Genomes, Kyoto University, Japan.