The transcriptional response of *C. albicans* to weak organic acids, carbon source and *MIG1* inactivation unveils a role for *HGT16* in mediating the fungistatic effect of acetic acid.

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ABSTRACT

Candida albicans is a resident fungus of the human intestinal microflora. Commonly isolated at low abundance in healthy people, C. albicans outcompetes local microbiota during candidiasis episodes. Under normal conditions, members of the human gastrointestinal microbiota were shown to keep C. albicans colonization under control. By releasing weak organic acids (WOAs), bacteria are able to moderate yeast growth. This mechanism displayed a synergistic effect in vitro with the absence of glucose in medium of culture, which underline the complex interaction that C. albicans faces in its natural environment. Inactivation of the transcriptional regulator MIG1 in C. albicans results in a lack of sensitivity to this synergistic outcome. To decipher C. albicans transcriptional responses to glucose, WOAs and the role of MIG1, we performed RNA sequencing on four biological replicates exposed to combinations of these 3 parameters. We were able to characterise the i) glucose response, ii) response to acetic and butyric acid, iii) MIG1 regulation of C. albicans and iv) genes responsible for WOAs resistance. We identified a group of 6 genes linked to WOAs sensitivity in a glucose-MIG1-dependent manner and inactivated one of these genes, the putative glucose transporter HGT16, in a SC5314 wild-type background. As expected, the mutant displayed a partial complementation to WOAs resistance in absence of glucose. This result points towards a mechanism of WOAs sensitivity in C. albicans involving membrane transporters which could be exploited to control yeast colonisation in human body niches.
INTRODUCTION

Considered as a human commensal, *Candida albicans* is frequently isolated from the human gastrointestinal (GI) tract where it co-exists harmlessly with its host. However, this yeast can become virulent particularly when associated with certain pathologies such as immunodepression or antibiotic treatment (Yapar 2014; Pfaller and Diekema 2007). At the core of *C. albicans* virulence is its dimorphic nature, an ability to switch between blastospore and filamentous forms, allowing *C. albicans* to invade tissue and evade immune cells (Saville et al. 2003; Krysan et al. 2014). This morphological switch of *C. albicans* occurs in response to numerous external stimuli such as temperature, pH, hypercapnia, serum, and mannitol or ammonium concentrations. Cell growth can also be impacted by as many conditions and demonstrates how responsive the yeast is to its environment. In its natural niches (GI tract, vagina, skin), some of these signals such as weak organic acids (WOAs) are metabolites originating from the local bacterial population. For example, *Lactobacillus*, predominant genus of the vaginal microbiota, produces lactic acid to control *C. albicans* colonisation in healthy women via WOAs acidification (Osset et al. 2001; Kohler et al. 2012). On another end, acetic and butyric acids are synthesized during anaerobic fermentation by bacteria present in the human GI tract (Mortensen and Clausen 1996) which are able to reduce the growth rate of *C. albicans in vitro* as well as decrease yeast GI colonisation in mice (Fan et al. 2015). Indeed, addition of 50mM of acetic acid to mice drinking water reduced by a factor of 10 the number of live *C. albicans* per gram of stool in antibiotic-treated mice. We and others have previously confirmed this negative impact of WOAs on *C. albicans* growth *in vitro* (Cottier et al. 2015a; Huang et al. 2011). We demonstrated that when exposed at concentrations of acetic and butyric acid similar
to the ones identified in human stool, *C. albicans* growth rate was reduced by around 50%. This triggered up-regulation of genes involved in iron transport and an opposite effect on RNA synthesis genes (Cottier et al. 2015a). We further identified the transcriptional regulator *MIG1* as a central contributor to WOAs resistance. *MIG1* inactivation increased sensitivity to acetic acid by 80% and by 20% for lactic and butyric acid in YPD media (Cottier et al. 2015b). In the model yeast, *Saccharomyces cerevisiae*, Mig1p is known as a major regulator involved in glucose repression. In presence of glucose, this zinc-finger protein represses the expression of genes encoding proteins involved in utilisation of non-glucose sugars such as maltose or sucrose. Similarly in *C. albicans*, inactivation of *MIG1* has been associated with derepression of gluconeogenic genes like *PCK1* (Murad et al. 2001). When *C. albicans* is grown on maltose as a main carbon source instead of glucose, a wild-type strain of *C. albicans* displays an increased sensitivity to WOAs by up to 75% for acetic and butyric acid (Cottier et al. 2015b). This mimics the phenotypes observed with the *MIG1* deletion mutant.

To identify which genes are involved in the *MIG1*- and/or glucose-dependent sensitivity to WOAs in *C. albicans*, we investigated the transcriptomic responses of this yeast to acetic and butyric acid in presence of glucose or maltose for a wild-type and *MIG1* mutant strain. Using RNA-sequencing, this multi-parametric design allowed us to characterise i) the glucose response, ii) the response to acetic and butyric acid, iii) the *MIG1* regulation of *C. albicans*, and iv) the *MIG1*- glucose-dependent genes involved in WOAs sensitivity. The latter represented a group of 6 genes whose expression is significantly altered between wild-type and *MIG1* mutant strains; but also between the different sources of carbon in presence of WOAs. We focused our attention on one of them, *HGT16*, a putative glucose transporter which displayed a strong correlation with cell growth rate. We demonstrated that inactivation of this gene induced a partial recovery to
acetic acid resistance in glucose deprived media. This multifactorial analysis put light on *C. albicans* responses to common environmental cues; and help investigate the synergistic effect of different signals on *C. albicans* growth. Such approach can support the development of innovative mechanisms to control *C. albicans* colonization.
MATERIALS AND METHODS

Strains and culture conditions

The wild-type *Candida albicans* strain SC5314 (Noble and Johnson 2005) and MIG1 mutant (Cottier et al. 2015b) were used throughout this study. Yeasts strains were grown in Yeast extract-Peptone-Dextrose (YPD) media (1% w/v yeast extract, 2% w/v peptone and 2% w/v D-glucose, supplemented with 1.5% w/v agar for solid media only) or YPM (identical composition to YPD except D-glucose was replaced by maltose) at 37 °C in a shaking incubator at 150 rpm. Stock cultures were preserved in 35% glycerol and maintained at −80 °C. Acetic and butyric acid (from either Merck or Sigma), were added to freshly re-launched overnight cultures, diluted down to an optical density (O.D.) at 600 nm of 0.1.

RNA sequencing

Cells were harvested by centrifugation and the pellet was flash-frozen in liquid nitrogen. RNA extractions and sequencing were performed identically to the previously described protocol (Cottier et al. 2015a). Raw and processed sequencing data are available on the Gene Expression Omnibus database (http://www.ncbi.nlm.nih.gov/geo/) under accession number GSE99767.

Data analysis

Reads were processed following the previously described protocol of Cottier *et al*. (Cottier et al. 2015a) using the *C. albicans* genome annotation (Assembly 21, version s02-m08-r09). Reads Per Kilobase per Million mapped reads (RPKM) were calculated using ANOVA in R (3.1.2) with ezANOVA (4.4-0). Statistical analysis was performed after addition to all values of the lowest RPKM measurement, then data were log10 transformed. Differentially expressed
transcripts were identified on Prism (GraphPad) with Two-stage linear step-up procedure of
Benjamini, Krieger and Yekutieli, with Q = 1%. Each genes were analyzed individually, without
assuming a consistent SD.

GO enrichment analysis was performed with the CGD Gene Ontology Term Finder (Inglis
et al. 2012), with p-values corresponding to Bonferroni-corrected hypergeometric test p-values.
The magnitude of the overlap between two gene lists was evaluated as the ratio of the
intersection over the union of the two lists. Statistical significance of the gene lists’ overlap was
evaluated by a series of a Fisher’s exact tests. All other statistical analyses were performed in
Microsoft Excel 2013 or Prism and, unless otherwise indicated in the respective figure legends,
we used unpaired t-tests assuming unequal variances and error bars corresponded to standard
erors of the mean.

Strains are available upon request. Supporting information file contains additional
descriptions of material and methods, figures and tables. File S1 contains RPKM values for each
genesis in all conditions. Gene expression data are available at GEO with the accession number:
GSE99767.
RESULTS

Wild-type and MIG1 mutant sensitivity to WOAs in presence or absence of glucose.

Our previous works (Cottier et al. 2015b; Cottier et al. 2015a) characterised the transcriptional response of C. albicans to WOAs in MRS media pH 5.5, and identified Mig1p as a general transcriptional factor involved in WOAs sensitivity in YPD media pH 5.5. The latter effect was dependent on the carbon source as yeast were more sensitive to WOAs when glucose was replaced by maltose in YPD media. We reproduced those results in YPD and YPM media pH 5.5 supplemented with and without acetic or butyric acid at their respective inhibitory concentration of 50% (IC₅₀) for both wild-type (SC5314) and mig1Δ strain. As expected, a growth rate reduction of around 50% was observed for the wild-type cells in presence of acetic or butyric acid compared to YPD alone (Fig. S1). This increased sensitivity to WOAs between the two strains was not observed when cells were grown in YPM media. These results are in agreement with our previous observation (Cottier et al. 2015b). MIG1 mutant displayed a significantly higher sensitivity to both acetic and butyric acid under the same condition than the control strain, respectively 75% and 20%. We also confirmed that SC5314 strain displayed a significantly higher sensitivity to acetic and butyric acid when grown in YPM compared to YPD media. As these results were in concordance with our previous published data (Cottier et al. 2015b), we collected cells at the end of the 6h growth assay and extracted RNA from the 12 conditions tested.

Transcriptional responses of C. albicans.

We prepared cDNA libraries from the total RNA extracted for all 48 independent samples (12 conditions and 4 biological replicates); sequencing was performed on an Illumina HiSeq
2000 platform using 2x50bp reads. We obtained an average of $1.4 \times 10^7 \pm 2.5 \times 10^6$ reads per sample, which were mapped to 6453 transcripts, including both open-reading frames and non-coding RNAs. After processing of the reads, we reported genes expression as RPKM values. Hierarchical clustering (Fig. 1) of the 48 samples displayed a clear separation principally to WOAs treatment, followed by carbon source (YPD or YPM) and finally strains. Replicates were closely clustered together (except for 4 samples of replicate 1) and indicative of a good reproducibility without outliers. To investigate the transcriptional responses to i) glucose (YPD vs YPM), ii) \textit{MIG1} (SC5314 vs \textit{mig1}Δ in YPD media), iii), acetic and butyric acid in YPD, iv) WOAs and carbon source simultaneously, we performed pair by pair comparisons. We obtained a list of genes significantly regulated between two conditions, and focused our attention on genes with at least a 2-fold regulation. For each comparison, we performed global functional analysis of these genes lists to gain biological insights into the transcriptional responses. We found statistically significantly enriched Gene Ontology (GO) Biological Process terms among the transcripts significantly up- or down-regulated.

**Glucose response**

\textit{C. albicans} is frequently grown in laboratory condition in rich media, like YPD, which contains 2\% glucose, but this concentration can be as low as 0.05\% in some body niches (e.g. bloodstream) (Rodaki et al. 2009) or absent and also subdued by more complex sugars like in the GI tract. To study the impact of an alternative carbon source on \textit{C. albicans}, we selected a close comparative: maltose, a disaccharide formed by two units of glucose which is produced in the gut during starch breakdown, and which alter the wild-type \textit{C. albicans} strain growth rate by only less than 10\% (Fig. S1). Additionally, maltose was shown in \textit{S. cerevisiae} to alleviate glucose repression almost as effectively as \textit{MIG1} inactivation (Klein et al. 1996). We first
compared the transcript profile between YPM and YPD media for the SC5314 strain; we identified 434 genes up-regulated and 817 down-regulated in presence of maltose (Fig. 2A). A result expected as Mig1p is a central regulator of glucose repression (Zaragoza et al. 2000). These numbers were respectively of 86 and 52 in a MIG1 mutant background, demonstrating that only 12% of genes regulated by glucose were mig1-independent. Down-regulated genes were significantly enriched in processes related to translation and cell cycle, as anticipated from the decrease in C. albicans growth rate when exposed to maltose and in agreement with previous data obtained in S. cerevisiae (Ashe et al. 2000). On the other end, among the up-regulated genes, ATP metabolism and monosaccharide transport were activated. Indeed, 11 transporter genes displayed a higher expression level (GAL1, HGT13, HGT17, HGT19, HGT6, HGT7, HGT8, HXK2, HXT5, RGT1, orf19.4090). Maltose transporter (MAL31) but also maltase (MAL32), and alpha-glucosidase (MAL2) were all up-regulated by a factor of at least 17. These regulation are indicative of an adaptation of C. albicans to an alternative carbon source.

Acetic and butyric acid responses

We formerly described the transcriptional response of C. albicans to lactic, acetic, butyric and propionic acid in MRS media, a glucose rich media (2%), at pH 5.5 (Cottier et al. 2015a). We now performed a similar analysis in YPD media with acetic and butyric acid at IC50 with the identical SC5314 strain. Compared to the control condition (YPD), addition of acetic acids to the media induced up-regulation of 1251 genes and down-regulation of 316, while these numbers were respectively of 895 and 202 for butyric acid (Fig. 2B). 50% of these genes shared a similar regulation with both acids, and displayed a significant enrichment in genes related to ATP synthesis and mitochondrial respiration. Despite the reduction in growth induced by the presence
of acids, *C. albicans* cells increased genes expression related to production of energy. In our previous study, these processes were similarly enriched in up-regulated genes at early time points. In this study, we also report a significant enrichment in up-regulated genes for the processes of ribosome biogenesis and RNA processing in YPD media, but which was observed to be strongly down-regulated in MRS media (Cottier et al. 2015a). To elucidate this discrepancy, we compared the results from this and previous study. Transcriptional profiles of SC5314 in MRS and YPD media with or without acetic or butyric acid were analysed, and we observed major differences between the two media (Fig. S2). Indeed, close to 61% of *C. albicans* genes were significantly regulated between MRS and YPD alone at pH 5.5. *C. albicans* gene expression was then strongly affected by the medium of culture, and by extension alters the transcriptional responses to WOAs. This observation might explain why, contrary to our previous dataset in MRS media (Cottier et al. 2015a), exposure to WOAs in YPD media did not revealed significant expression of genes encoding iron transporter. Under those conditions, only 52 genes were similarly regulated in response to acetic acid addition to the media of culture (MRS or YPD); and in agreement with our previous analysis, genes involved in arginine biosynthesis (*ARG1, ARG3, ARG5,6, CPA1, CPA2*) were up-regulated in both media.

**Mig1 response**

We previously demonstrated the central role of Mig1p in weak organic acids sensitivity, as the *MIG1* mutant showed a decreased growth rate in response to lactic, acetic, propionic and butyric acids when compared to the SC5314 control strain (Cottier et al. 2015b). To investigate which Mig1p targets were involved in this phenotype, we first characterized the transcriptional profile of the *MIG1* mutant against SC5314 control strain in YPD media. Inactivation of the
transcription factor lead to up-regulation of 64 genes and down-regulation of 25 (Fig. 2C). As Mig1p has been described by others to be a genes repressor (Zaragoza et al. 2000; Murad et al. 2001), it was expected that a higher number of genes would be up-regulated in the mutant strain. This group was enriched in genes involved in monosaccharide transporter (GAL1, HGT1, HGT16, HGT19, HGT2, HGT6, HGT7, HXK2, HXT5) and alternative carbon source utilization like galactose. Similarly to S. cerevisiae, Mig1p in C. albicans is responsible for the repression of hexose membrane transporter in presence of glucose (Ozcan and Johnston 1999). In S. cerevisiae, Mig1p is known to be phosphorylated by Snf1p in absence of glucose prompting a displacement of Mig1p from the nucleus to the cytoplasm and relieving its repression of genes (Treitel et al. 1998; De Vit et al. 1997). Thus, as in the absence of glucose where Mig1p is localized outside the nucleus, a mig1Δ strain should provide a similar profile of expression as a wild-type strain in YPM media. Indeed, only 2 genes were observed as differentially expressed between the two strains in presence of maltose. This result is reproduced phenotypically as no significant difference in growth rate is observed between SC5314 and mig1Δ strain in any condition involving YPM media (Fig. S1).

Genes involved in Mig1p- glucose- dependent WOAs resistance

We observed that inactivation of MIG1 induced a higher sensitivity to WOAs in YPD media compared to the control strain, and similarly that wild-type strain SC5314 displayed a significant lower growth rate in presence of WOAs while grown in YPM versus YPD media (Fig. S1). As the latter effect is absent in a MIG1 mutant, we hypothesized that Mig1p targets involved in WOAs sensitivity were also regulated between YPD and YPM supplemented with WOAs in SC5314 background. We performed pair by pair comparisons between the transcript
profiles of wild-type strain grown in YPD with acetic acid versus YPM with acetic acid, and
identically with butyric acid. 93 genes were up-regulated in presence of acetic acid and 174
down-regulated. These numbers were 579 and 374 respectively for butyric acid (Fig. 2D). As
expected, in presence of maltose, processes involved in glucose metabolism were down-
regulated with both acids. A total of 138 genes were similarly regulated by both conditions.

Our final objective was the identification of genes involved in WOAs sensitivity, where the
impact on the phenotype is further increased by inactivation of \textit{MIG1} or in absence of glucose.
Using our multifactorial analysis, we now can compare the list of genes significantly regulated
by i) WOAS, ii) \textit{MIG1}, iii) carbon source during WOAs exposure. As previously stated, \textit{C. albicans}
exposure to acetic or butyric acid led to the common regulation of 885 genes, while
Mig1p was responsible for the regulation of 89 genes. Finally, we identified a set of 147 genes
which expression is altered between YPD and YPM media in presence of WOAs. By comparing
these three lists (Fig. 3A), we noticed an intersect for only 6 genes (\textit{GLG2}, \textit{orf19.7566}, \textit{ALD6},
\textit{FDH1}, \textit{HGT16}, \textit{orf19.94}). All 6 genes were up-regulated under conditions where \textit{C. albicans}
sensitivity to WOAs was enhanced, implicating that the functions of these genes is associated
with increasing sensitivity to WOAs rather than inhibition of resistance mechanism. We
performed a correlation analysis between profile of expression of these 6 genes and the growth
rate reported for each condition (Fig. 3B). As expected, we obtained negative Pearson coefficient
factors between the two set of data, and all correlation were significant (p < 0.05), except for
\textit{ALD6}. Interestingly, the strongest coefficient factors were obtained for \textit{HGT16} and \textit{orf19.7566},
at -0.874 and -0.872 respectively. Both genes encode for putative transmembrane protein with 11
transmembrane domains. Due to their transmembrane localisation, these protein might have an
impact on plasma membrane integrity and molecular diffusion.
**HGT16 inactivation restores acetic acid resistance.**

Following identification of putative targets involved in Mig1p-glucose-dependent WOAs sensitivity, we hypothesize that cell membrane transporters could actively or passively transport WOAs, potentially enhancing their impact on cellular processes. Therefore, as a proof of concept, we decided to inactivate the putative glucose transporter of the major facilitator superfamily: \textit{HGT16} (\textit{orf19.6141}). Both alleles of \textit{HGT16} were inactivated using the SAT1-flipping strategy in SC5314 background (Sasse and Morschhauser 2012). We confirmed by flow cytometry that no aneuploidy were introduced during inactivation process (Fig. S3A) and no detection of \textit{HGT16} RNA messenger was possible in the double mutant: \textit{hgt16Δ} (Fig. S3B). We then grew SC5314 and \textit{HGT16} mutant strain in YPD or YPM media supplemented with or without acetic acid, and measured growth rate over a 6h period. We confirmed by qRT-PCR, the observation made with RNA-seq, that \textit{HGT16} expression is significantly increased by at least a 4-fold factor by acetic acid when assayed in YPM media (Fig. S3A-B), but constant between YPD and YPM in absence of acetic acid. As expected by its low level of expression in YPD and YPM alone, \textit{HGT16} inactivation did not significantly alter growth rates between those conditions. However, \textit{HGT16} mutant displayed a 5-fold increase in acetic acid resistance compared to control strain in YPM complemented with acetic acid, while an increase of 33% was observed with YPD media, but was not significant (Fig. 4). These results thus confirms that \textit{HGT16} up-regulation during WOAs exposure might contribute to WOAs sensitivity in \textit{C. albicans}. 
DISCUSSION

Natural niches of *C. albicans* are complex environments where nutrients composition and availability are unpredictable and competition with other organisms for resources are strong (GI tract, skin). Mechanisms of adaptation to these conditions are critical for the yeast survival (Miramon and Lorenz 2017). Indeed, *C. albicans* has been described to respond to a large array of stimuli from carbon dioxide (Cottier et al. 2012) to substrate topography (Brand et al. 2008), passing by a large range of molecule like pheromones, carbon source or ammonium (Cottier and Muhlschlegel 2009). Most of these studies were focused on a single perturbation to the media of culture, while *C. albicans* is most likely facing several changes concomitantly in its natural environment (e.g. human diet). Integration of these different and sometimes contradictory signals remains an important part of understanding mechanisms of *C. albicans* adaptability to its environment. Here, by studying *C. albicans* response to WOAs in different media and genetic backgrounds, we aimed to identify the core components of this yeast sensitivity to weak organic acids. Our previous work (Cottier et al. 2015b) pointed out to conditions where sensitivity to WOAs is enhanced without changing acids concentration but by acting on other parameters such as carbon source (maltose instead of glucose) or inactivation of genes (*MIG1)*.

Glucose response of *C. albicans* had been described by Rodaki *et al.*, as the authors performed transcriptional profile of yeast grown in lactate compared to a range of glucose levels, from 0.01 to 1.0%, by microarray analysis (Rodaki et al. 2009). Similarly, we observed an increase in genes related to aerobic respiration in absence of glucose, but from the 180 genes (expression ratio ≥ 2) identified as regulated between lactate and 1% glucose, only 20 were commonly present in our analysis. While both studies used derivate media from YPD, strain of
C. albicans (THE1 versus SC5314), time of incubation (30 min versus 6 hours), temperature (30°C versus 37 °C) and alternative carbon source (Lactate vs Maltose) were all different. Those differences could explain the lack of cohesion between the two approaches. We reached a similar situation when comparing our transcriptional response of C. albicans to acetic and butyric acid in MRS and YPD media. Strains and condition of culture were identical between the current study and our previous dataset (Cottier et al. 2015a), but we noticed only few common responses like ATP synthesis or arginine biosynthesis, and dramatic differences particularly regarding ribosome biogenesis. In fact, 61% of all C. albicans genes were significantly regulated between MRS and YPD alone, both rich media with 2% glucose, in a SC5314 strain. This high level of gene expression alteration between control samples explained the limited overlap between transcriptional profiles of C. albicans in response to WOAs.

Our transcriptional profile on MIG1 inactivation presented closer similarities with previous studies. A macroarray covering 2002 out of the 6218 open reading frames from C. albicans was performed by Murad et al., where they described that 0.8% of the genes (fold change ≥ 3) were regulated by Mig1p (Murad et al. 2001) in YPD media at 30 °C. In our study, using an SC5314 background strain at 37 °C, we reported 0.74% of total genes similarly regulated. Some of the principal expressed genes were identified in both studies (HGT1, GCA1, GAL1, orf19.11, orf19.6888, PHR1, MDH1). We also confirmed derepression of a major gluconeogenesis enzyme, the phosphoenolpyruvate carboxykinase PCK1, in mig1Δ strain as previously described (Murad et al. 2001). In agreement with the described function of Mig1p in S. cerevisiae (Garcia-Salcedo et al. 2014), we reported that inactivation of MIG1 lead to a general derepression of genes involved in non-glucose utilisation like monosaccharide transporters (GAL1, HGT1, HGT16).
From the 90 genes significantly regulated by Mig1p, we identified 36 which were also differently expressed in presence of acetic or butyric acid in YPD media (Fig. 2C). To reduce further the number of putative Mig1p target involved in WOAs sensitivity, we compared the transcriptional profile of genes differently regulated between YPD and YPM media in presence of acids. We noticed that in presence of YPM, where Mig1p is inactive, *C. albicans* SC5314 strain displayed a higher sensitivity to WOAs compared to YPD media. Similarly an increase sensitivity to WOAs is observed between SC5314 and MIG1 inactivated strain in YPD media. We ended with a list of 6 genes (*GLG2, orf19.7566, ALD6, FDH1, HGT16, orf19.94*) which expression was negatively correlated with cells growth rate. None of these genes were part of the stress core responses to WOAs previously identified (Cottier et al. 2015a), confirming the absence of a general stress response in *C. albicans* as demonstrated by Enjalbert et al. (Enjalbert et al. 2003). Our hypothesis that inactivation of one of these genes would induce a higher resistance to WOAs was validated with *HGT16*. The mutant strain remained sensitive to WOAs exposure, but show a 5-fold increase in resistance to acetic acid compared to SC5314 wild-type strain in YPM media. The question persists to assess the remaining Mig1p targets for their role in WOAs resistance. *Orf19.7566* encoding another predicted membrane transporter would be a prime choice, following the results obtained with *HGT16*. We could hypothesised that these membrane transporters might open channels for the WOAs molecules to be transported inside the cells increasing their impacts on metabolic pathways.

In this study, we demonstrated an ability to reduce *C. albicans* growth rate by altering cellular compound (*MIG1* mutant) or external cues (carbon source). We also demonstrated that expression of certain protein like Hgt16p are, at least partially, responsible for *C. albicans* sensitivity to WOAs. Mechanisms to target increased expression of such genes, or alteration in
carbon source in *C. albicans* environment might be promising methods to keep yeast growth and colonization under control.

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FIGURE LEGENDS

**Figure 1. Global transcriptional response of *C. albicans* to carbon source, WOAs and *MIG1* inactivation.** The figure displays a two-way hierarchical clustering of 6,218 transcripts (rows) and 48 samples (columns). Log10-transformed RPKM expression values were converted to z-scores, with red indicating expression levels above and blue symbolizing expression levels below the mean expression level of each gene across the samples. Distance metric = 1 – Pearson correlation.

**Figure 2. Significantly regulated genes in response to different growth condition.**

(A) Genes significantly regulated in response to glucose and maltose. Up- and down-regulated genes obtained by comparison of transcriptional profiles between YPM over YPD media for SC3514 and *mig1Δ* strain. (B) Genes significantly regulated in response to WOAs. Up- and down-regulated genes obtained by comparison of transcriptional profiles between YPD supplemented with acetic or butyric acid over YPD media for SC3514 strain. Not represented: 1 gene (*orf19.3684*) displayed opposite regulation between acetic acid (down-regulation) and butyric acid (up-regulation), and one with the opposite trend (*orf19.7042*). (C) Genes significantly regulated in *MIG1* mutant. Up- and down-regulated genes obtained by comparison of transcriptional profiles between *mig1Δ* over SC3514 strain in YPD and YPM media. (D) Genes significantly regulated in response to carbon source in presence of WOAs. Up- and down-regulated genes obtained by comparison of transcriptional profiles between YPM supplemented with acetic or butyric acid over YPD media supplemented with identical acids for SC3514 strain.
Not represented: 9 genes displayed opposite regulation between acetic acid (down-regulation) and butyric acid (up-regulation).

**Figure 3. Identification of MIG1-glucose-dependent genes involved in WOAs sensitivity.**

(A) Comparison of genes found significantly regulated by Mig1p (89), presence of WOAs in YPD media (885), and carbon source in presence of WOAs (147). A group of 6 genes was identified as commonly present in all 3 lists. (B) Mean of the RPKM values plotted according to mean growth rates of the 4 replicates per condition for GLG2, orf19.7566, ALD6, FDH1, HGT16 and orf19.94. Solid black lines represent linear regression. Grey lines represent SEM.

**Figure 4. HGT16 inactivation increases acetic acid resistance.**

(A) Ratio of RPKM value from HGT16 over ACT1 obtained by RNA-sequencing in indicated media for SC5314. *p < 0.05 (B) Proposed model where glucose via MIG1p inactivate HGT16 expression, which is itself stimulated by the presence of WOAs in growth medium. Production of HGT16p could increase WOAs import which lead to a reduction in *C. albicans* growth rate.
**Carbon source regulation**

Up-regulated in YPM vs. YPD:
- Oxidation-reduction process (p=1.07e-10)
- ATP metabolic process (p=2.54e-6)
- Purine nucleoside triphosphate metabolic process (p=5.55e-6)
- Monosaccharide transport (p=3.2e-4)
- Aerobic respiration (p=5.9e-4)

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ATP metabolic process (p=5.17e-06)

Down-regulated in YPM vs. YPD:
- Translation (p=1.22e-37)
- Peptide metabolic process (p=1.01e-31)
- DNA replication (p=3.01e-19)
- Mitotic cell cycle (p=1.12e-6)
- Chromosome segregation (p=5.13e-5)

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**Weak organic acid regulation**

Up-regulated in presence of WOAs vs. YPD alone:
- Mitochondrial transport (p=1.10e-10)
- Peptide metabolic process (p=2.46e-07)
- Mitochondrial RNA metabolic process (p=5.57e-05)
- ATP synthesis coupled proton transport (p=1.58e-05)

512

Fatty acid oxidation (p=2.9e-04)

Down-regulated in presence of WOAs:
- DNA replication (p=1.45e-06)
- Mitotic sister chromatid cohesion (p=4.07e-06)

170

Oligopeptide transmembrane transport (p=5.9e-04)

**Mig1 regulation**

Up-regulated in mig1Δ vs. SC5314:
- Monosaccharide transport (p=2.15e-09)
- Galactose catabolic process (p=7.61e-05)

64

Down-regulated in mig1Δ vs. SC5314:
- Response to inorganic substance (p=5e-3)

24

**Carbon source regulation in presence of WOAs**

Up-regulated in YPM vs. YPD, both supplemented with WOAs:
- IMP metabolic process (p=1.26e-05)
- Sister chromatid cohesion (p=1.14e-05)
- Fatty acid oxidation (p=2.5e-04)

509

Organic acid catabolic process (p=6.42e-07)

Down-regulated in YPM vs. YPD, both supplemented with WOAs:
- Glucose metabolic process (p=1.35e-09)
- Glycolytic process (p=9.07e-08)
- ATP generation from ADP (p=9.07e-08)
- Gluconeogenesis (p=1.39e-05)

297

Response to arsenic-containing substance (p=5e-03)

Carbohydrate metabolic process (p=4.34e-05)
**Mig1 regulated genes**

(mig1Δ vs. SC5314 in YPD)

- 43 genes
- 30 genes
- 809 genes

**WOAs regulated genes**

(YPD+WOAs vs. YPD in SC5314)

- 6 genes
- 10 genes
- 40 genes

**Carbon source regulated genes (in presence of WOAs)**

(YPM+WOAs vs. YPD+WOAs in SC5314)

- GLG2
- orf19.7566
- ALD6
- FDH1
- HGT16
- orf19.94

**Correlation Analysis**

- **GLG2**
  - $r = -0.6518$
  - $p$-value $= 0.0216$

- **orf19.7566**
  - $r = -0.8727$
  - $p$-value $= 0.0002$

- **ALD6**
  - $r = -0.4444$
  - $p$-value $= 0.1476$

- **FDH1**
  - $r = -0.5922$
  - $p$-value $= 0.0425$

- **HGT16**
  - $r = -0.8742$
  - $p$-value $= 0.0002$

- **orf19.94**
  - $r = -0.7499$
  - $p$-value $= 0.005$