1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22

Sequencing and *de novo* assembly reveals genomic variations associated with differential responses of *Candida albicans* ATCC 10231 towards fluconazole, pH and non-invasive growth

Authors

Gajanan Zore^{1#}, Archana Thakre¹, Rajendra Patil², Chaithra Pradeep³, Bipin Balan³

Affiliations

¹Research Lab. 1, School of Life Sciences, Swami Ramanand, Teertha Marathwada University (SRTMU), Nanded-431606 (MS) India.

² Department of Biotechnology, Savitribai Phule Pune University (SPPU), Pune-7 (MS) India.

³SciGenom Labs Pvt Ltd., Plot No. 43A, SDF 3rd Floor, CSEZ Kakkanad, Cochin, Kerala 682037.

Abstract

The whole genome sequencing generated 8.09 million paired-end reads, which were assembled into 2,262 scaffolds totaling 17,113,050 bp in length. We predict 7,654 coding regions and 7,647 genes, and annotated the coding regions with gene ontology terms based on similarity to other annotated genomes. Genome comparisons revealed variations (including SNP and indels in genes involved in pH response, fluconazole resistance and invasive growth) compared to *C. albicans* SC5314 and WO1.

Key Words: *Candida albicans* ATCC10231, Whole genome sequencing, Illumina-HiSeq2500, Fluconazole resistance, pH non responsive, Non-invasive.

63

64

65 *Candida* species are important opportunistic pathogens, especially of
 66 immunocompromised individuals, where they exhibit high mortality rates (40-75%)¹⁻
 67 ⁶. The ability of these species to change morphophysiology according to microenvi-
 68 ronment contributes to their success as opportunistic pathogens¹⁻⁴. Considering its
 69 clinical and economic impact, the Centers for Disease Control and Prevention (CDC),
 70 in consultation with experts from NIH and the US-FDA, put *Candida* in the list of or-
 71 ganisms that pose a serious antibiotic resistance threat⁵.

72 *C. albicans* ATCC 10231(Robin) Berkhout was originally isolated from a man with
 73 bronchomycosis is included in the *Candida albicans* Drug Resistance Panel
 74 (ATCC®MP8™) recommended by the ATCC for use as a control in ASTM Standard
 75 Test Method E9799 (ATCC®MP8™)¹⁻⁷. In addition to FLC resistance, it also exhibits
 76 traits like pH non-responsiveness and non-invasive growth⁸⁻¹⁰. Before sequencing, dif-
 77 ferential responses viz. fluconazole resistance, pH non-response and non-invasive
 78 growth was confirmed in our laboratory⁷⁻¹⁰. To understand the molecular basis of the-
 79 se traits, we have sequenced the whole genome, developed a *de novo* assembly and
 80 compared it with the reference genomes of *C. albicans* SC5314 and WO1. *Candida*
 81 *albicans* (MTCC227), a type strain of *C. albicans* ATCC10231, was collected from
 82 Microbial Type Culture Collection (MTCC), Institute of Microbial Technology,
 83 Chandigarh (India)¹. High-quality genomic DNA was extracted by lysing *C. albicans*
 84 cells grown in yeast extract peptone dextrose broth for overnight at 28°C with lyticase
 85 and then alkaline lysis method¹¹. An intact band on the agarose gel and 1.82 ratio of
 86 OD at 260/280 confirmed the quality of genomic DNA preparation. Concentration
 87 assessed using nano drop was found to be 141.9 ng/micro L. A library of 8.09 million
 88 short insert paired end (2x100) reads of 1.6 Gb total size, with an average insert size

89 of 320 bp was generated using Illumina HiSeq2500 platform by following a standard
90 Illumina protocol (Illumina Inc., Cat. # PE-930–1001) as per manufacturer’s instruc-
91 tions¹²⁻¹⁵ (Table 1, Data Citation 1, 2). Read quality was assessed using base quality
92 score distribution, sequence quality score distribution, average base content per read,
93 GC distribution in the reads, PCR amplification issue, check for over-represented se-
94 quences and adapter trimming to exclude low-quality sequence reads (the FastQC ver-
95 sion v0.10.1)¹²⁻¹⁵. The numbers of bases trimmed from the 5' and 3' end of Read1(R1)
96 were 9 and 5 respectively; while that of Read2 (R2) at 5' and 3' end were 10 and 4 re-
97 spectively¹²⁻¹⁵. The adapter sequences were removed using cutadapt (Version-1.8.1)¹².
98 *De novo* assembly of raw reads was performed using MaSuRCA (Version-3.1.3) as
99 per Zimin et al. (2013); while processed reads were assembled using SOAPdenovo2
100 and SPAdes as mentioned in Luo, et al. (2012) and Bankevich et al. (2012) respective-
101 ly¹⁸⁻²⁰. Paired-end reads were assembled into 2,262 scaffolds of an average length of
102 7,565.45 bp and a total length of 17,113,050 bp using MaSuRCA (Table 1, Data Cita-
103 tion 1, 2; Table 2). While using SPAdes (version 3.6.1), we could assemble paired end
104 reads in to 6339 contigs with average length of 2421.19 bp and total length of
105 15347969 bp and that of using SOAPdenovo263mer were 28492 contigs with average
106 length of 667.44 bp and total length of 19016774 bp (Table 2). We observed that
107 MaSuRCA performed better than other assemblers for the sample sequenced in this
108 study and it could be due to the intrinsic characteristic of two algorithm (DBG and
109 OLC) merged together¹⁶. MaSuRCA computes an optimal k-mer size of 71 based on
110 the read data and GC content¹⁶. To validate the assembly further, high-quality reads
111 were aligned to MaSuRCA assembled genome using BWA, which resulted in 83.57%
112 alignment¹⁸⁻²⁰.
113 We predict a total of 7,654 coding regions (CDSs) and 7,647 genes from the scaffolds
114 using AUGUSTUS (version 2.5.5) program as per Stanke et al. (2004)²¹.(Table 1, File

2-3, Data Citation 1). Predicted genes were annotated using Perl scripts program using NCBI database and BLASTX program and annotated according to organisms, genes and proteins to the matched genes, gene ontology, and pathways²²⁻²⁴ BLASTX hits from the CDS identified 32 organisms (Fig. 1; Table 1, Data Citation 1). The top hit was found to be *C. albicans*, and 99 percent of the genes identified were homologous to *C. albicans* (Fig. 2; File 8, Data Citation 1). Functional annotation terms were predicted for the CDSs from the biological process, molecular function and cellular component branches of the Gene Ontology (GO) (Fig. 3; File 7, Data Citation 1).

The variant calling performed for the test genome against the reference genomes of *C. albicans* as well as our *de novo* assembled genome using SAMtools mpileup program revealed global variation, i. e. 35,709 and 94,627 variants compared to *C. albicans* SC5314 and WO1 respectively (Table 3-7)²²⁻²⁵. The identified variants were filtered using the cutoffs (read depth ≥ 10 and quality score ≥ 50)²⁵. The details of variants are provided in the table 6. The effect of the identified variants was predicted using SnpEff tool (version 4.3i) against the database created using the assembled genome and its annotation file²⁵. The variants identified overall suggest that *C. albicans* ATCC10231 is closer to SC5314 than WO1 (Table 3-7, Fig. 4).

Polymorphic sites within the assembled genome were identified by mapping the reads to reference genomes of *C. albicans* SC5314 (Data Citation 1). Nine genes (viz.VPS36, RIM21, RIM9, CSR1, PHR1, RIM13, vps28, SIT4,CCC1/4) involved in pH response, seven (viz. ERG6, ERG11, CDR3, POR1, CDR4, NDT80, HOG1) in fluconazole resistance and forty (including ENO1, PHR1, CDC10, RSP5, SET1, GPR1, SIT4 and ASH1) in invasive growth showed significant variation (Table 8)^{4-6,8-10}. These variants could be responsible for pH non-responsiveness, fluconazole resistance and non-invasive growth in *C. albicans* ATCC10231 (Table 7, Supplementary Table S1-S7, Data citation 1)²⁶⁻³⁹.

Neutral pH response is mediated by a seven domain containing transmembrane receptor, DFG1 that along with other proteins including Vps36 (ESCRT II protein sorting complex subunit), Vps28 (ESCRT I protein sorting complex subunit) undergo endocytosis and associate with Endosomal Sorting Complex Required for Transport (ESCRT)²⁷⁻²⁹. Endocytosed Vps36 and Vps28 activate (phosphorylation and ubiquitination) Rim8 (β -arrestin like protein) that in turn interacts and recruit Rim21 (plasma membrane pH-sensor) and Rim101 (zinc finger transcription factor) at ESCRT complex²⁷⁻²⁹. This interaction brings RIM101 in proximity to RIM13 (protease) that cleaves C-terminal peptide and activate short N-terminal, zinc finger transcription factor, Rim101²⁷⁻²⁹. RIM101 recognizes 5'- NCCAAG-3' sequence and induce expression of alkaline pH responsive genes like PHR1 (cell surface glycosidase), Rim101 etc., under neutral pH²⁷⁻²⁹. The genes of *C. albicans* ATCC10231 involved in this process was found to exhibit the significant variation (SNP/indel) viz. VPS36 (32), VPS28 (4), RIM9 (36),13 (7) and 21 (1), CSR1 (16), PHR1 (7) and SIT4 (7). These variations could be associated with pH nonresponsiveness of *C. albicans* ATCC10231, as mutants were reported to affect alkaline pH induced hyphae formation and thus virulence²⁷⁻²⁹.

Activation of drug efflux pumps (MDR, CDR), modification and or over expression of drug target are the major mechanisms that confer drug resistance in both pro and eukaryotic organisms/cells²⁶. Our data suggest that FLC resistance in *C. albicans* ATCC10231 could be due to either modification in 14-alpha Demethylase (FLC target encoded by ERG11) and or drug efflux pumps²⁶. As seven genes involved in ergosterol biosynthesis (ERG11, ERG6), drug efflux and regulator (CDR3, CDR4, NDT80), stress response (HOG1) and a mitochondrial membrane transporter (POR1) exhibit variations (SNP/Indel)²⁶. However, it was reported that neither mutations nor

over expression of both CDR3 and CDR4 modulate FLC susceptibility, indicating that modified ERG11 could be conferring FLC resistance in *C. albicans* ATCC10231.

In addition to these, forty genes regulating the invasive growth of *C. albicans* ATCC 10231 exhibit significant variations (SNP/Indel) (Table 7, Supplementary Table S5-7, Data citation 1). SNPs were ranged from 1-47 i.e. maximum was observed in Nam2 (47) followed by SMC5 (42) and RIM21 (36) (Table 7, Supplementary Table S5-7, Data citation 1). Genes like ENO1, PHR1, CDC10, RSP5, SET1, GPR1, SIT4 and ASH1 reported to be essential for tissue invasion exhibit 7, 7, 4, 11, 4, 20, 7, 4 SNPs respectively (GPR1 and ASH1 exhibit 1 and 2 indels respectively) (Table 7, Supplementary Table S5-7, Data citation 1)³⁰⁻³⁹. Eno1 is a cell surface, plasminogen binding protein essential for tissue invasion³⁷. PHR1 codes for a glucosidase (involved in beta 1,3 glucan processing) is also required for maintaining hyphal morphology, adhesion (by regulating expression of Hwp1 and ECE1) and thus tissue invasion²⁷. A septin encoded by CDC10 is essential for invasive growth as mutants though form hyphae failed to penetrate solid surfaces both *in vivo* and *in vitro* and thus affect virulence³⁰. NEDD4 family E3 ubiquitin ligase coded by RSP5 is essential in systemic kidney infection in mouse³⁵. SET1, an H3K4 methyltransferase is essential for pathogenesis as loss of SET1 affected cell surface chemistry and adherence thus impacting epithelial cell invasion³⁸. GPR1 mutants failed to induce invasive growth on solid hypha-inducing media³⁹. SIT4 regulates two hyphae specific glucanases involved in cell wall biogenesis as mutants failed to induce invasive growth.

Whole genome sequencing of a strain differentially responsive to pH, invasive growth and anti-fungal agents has provided an insight into the molecular basis of these phenotypes. Thus it may find use as a unique reference database to understand novel mechanisms and survival strategies evolving in eukaryotic organisms in general and *C. albicans* in particular.

192 Data Records

193 The whole genome sequence of *Candida albicans* ATCC10231 is deposited to NCBI
 194 Sequence Read Archive (SRA) database as the raw FastQ files, whole genome assem-
 195 bly and annotation under accession number SRP067106 and Figshare.
 196 <https://doi.org/10.6084/m9.figshare.5349937.v1>. Data files available at Figshare are as
 197 follows:

198 **Table 1. Data Records**

Source	Protocol 1	Protocol 2	Protocol 3	Protocol 4 (Bioinformatic analysis)	Samples (Supplementary files)	Reference
Candida albicans	DNA isolation	Library preparation	Illumina HiSeq DNA sequencing	De novo genome assembly	File 1_genome_scf.fasta	1
				Gene prediction	File2_augustus.gff3	2
				CDs	File3_coding_seq.fa	
				Annotation using Uniprot	File4_Blastx_Uniprot_info.xls	
				Annotation	File5_NoBlastxresults.xls	
				Annotation using databases other than Uniprot	File6_BlastxResults_without_Uniprot_Annotation	
				Gene ontology	File7_GO_Annotation.xls	
				Organism annotation	File8_OrganismCount.xls	
Genome comparison with reference genome 1 (<i>C. albicans</i> SC5314) and Reference Genome 2 (<i>C. albicans</i> WO1)				Annotation	File 9. Annotated_variants.txt	
				Annotation	File 10. YEASTWGS_sample1_filtered.vcf	
				pH responsive genes	Table S1_phenotype-pH-resistance_CGDdb.txt	
				Non-invasive genes	Table S2_phenotype-invasive-growth_CGDdb.txt	
				Common genes (De novo)	Table S3_Denovo_commongene_annotation	
				Common genes (reference genes)	Table S4_Reference_commongene_annotation	

	Variants associated with differential responses	Table S5_Flucanazole-gene-variant-effect.txt	
		Table S6_pH-gene-variant-effect.txt	
		Table S7_Invasive-growth-gene-variant-effect.txt	

Acknowledgements

Authors are thankful to SERB, Govt. of India, India for financial assistance to GBZ under SERB FTSYS and Savitribai Phule Pune University, Pune (MS) India for financial assistance to RP. Authors are also thankful to Prof. Pandit Vidyasagar, Vice Chancellor, SRTM University, Nanded (MS) India for his encouragement and constant support.

Author contributions

GBZ conceived, designed the experiment analyzed the data, evaluated the conclusions and wrote the paper. RP performed the experiments. BB and CP performed bioinformatics analysis and evaluated conclusion. The manuscript is read and approved by all the authors before communication.

Competing interests

There is no competing interest about the data.

References

1. Arendrup MC. 2013. *Candida* and Candidaemia: Susceptibility and Epidemiology. Dan. Med. J. 60:B4698.
2. Kullberg BJ, Arendrup MC. 2015. Invasive candidiasis. New Eng J Med 373:1445-1456.
3. Eggimann P, Bille J, Marchetti O. 2011. Diagnosis of invasive candidiasis in the ICU. Ann Inten Care 1:1-37.

- 223 4. Biswas S, Van Dijck P, Datta A. 2007. Environmental sensing and signal trans-
 224 duction pathways regulating morphopathogenic determinants of *Candida albicans*.
 225 Microbiol Mol Biol Rev 71:348-76.
- 226 5. CDC report. Antibiotic Resistance Threats in the United States 2013. United
 227 States Dept. of Health and Human Services, CDC. 1-114 (2013).
 228 <https://www.cdc.gov/drugresistance/pdf/ar-threats-2013-508.pdf>
- 229 6. Jensen RH, Astvad KMT, Silva LV, Sanglard D, Jørgensen R, Nielsen KF,
 230 Mathiasen EG, Doroudian G, Perlin DS, Arendrup MC. 2015. Stepwise emer-
 231 gence of azole, echinocandin and amphotericin B multidrug resistance *in vivo* in
 232 *Candida albicans* orchestrated by multiple genetic alterations. J Antimicrob
 233 Chemother 70:2551-5.
- 234 7. ATCC *Candida albicans* Drug Resistance Panel. Use of the *Candida albicans*
 235 drug resistance (CADR) Panel (ATCC®MP8™) in anti-fungal drug testing. Amer-
 236 ican Type Culture Collection PO Box 1549 Manassas, VA 20108 USA
 237 <https://www.atcc.org/products/all/MP-8.aspx> (2016).
- 238 8. Zore GB, Thakre AD, Rathod V, Karuppayil SM. 2011. Evaluation of anti-
 239 *Candida* potential of geranium oil constituents against clinical isolates of *Candida*
 240 *albicans* differentially sensitive to fluconazole: inhibition of growth, dimorphism
 241 and sensitization. Mycoses 54:e99–109.
- 242 9. Devkotte AN, Zore GB, Karuppayil SM. 2005. Potential of plant oils as inhibitors
 243 of *Candida albicans* growth. FEMS Yeast Res. 5:867–73.
- 244 10. Kodgire, S. S. *Proteomic analysis of Candida albicans hyphae (filamentous and*
 245 *invasive) induced by neutral pH and identification of differentially expressed pro-*
 246 *teins*. Ph. D. Thesis in Biotechnology, SRTM University, Nanded (MS) India
 247 (2016).

- 248 11. RNeasy Mini Handbook - (EN) (4 th ed). RNeasy Mini Kit For purification of to-
249 tal RNA from animal cells, animal tissues, bacteria, and yeast, and for RNA
250 cleanup. 1-80 (2012).
251 [https://www.qiagen.com/ch/resources/resourcedetail?id=14e7cf6e-521a-4cf7-](https://www.qiagen.com/ch/resources/resourcedetail?id=14e7cf6e-521a-4cf7-8cbc-bf9f6fa33e24&lang=en)
252 [8cbc-bf9f6fa33e24&lang=en](https://www.qiagen.com/ch/resources/resourcedetail?id=14e7cf6e-521a-4cf7-8cbc-bf9f6fa33e24&lang=en).
- 253 12. Martin M. 2011. Cutadapt removes adapter sequences from high-throughput se-
254 quencing reads. EMBnet journal 17(1):10-12.
- 255 13. Li H, Durbin R. 1993. Fast and accurate short read alignment with Burrows-
256 Wheeler transform. Bioinformatics 25:1754-1760.
- 257 14. Joshi NA, Fass JN. 2011. Sickel: A sliding-window, adaptive, quality-based trim-
258 ming tool for FastQ files (Version 1.33) [Software] (2011). Available at
259 <https://github.com/najoshi/sickle>
- 260 15. Xu H, Luo X, Qian J, Pang X, Song J, Qian G, Chen J, Chen S. 2012. FastUniq: A
261 Fast *De Novo* Duplicates Removal Tool for Paired Short Reads. PLoS ONE 7:
262 e52249.
- 263 16. Zore GB. 2016. Whole genome sequence of *Candida albicans* ATCC10231.
264 *NCBI* *Sequence* *Read* *Archive*
265 SRP067106.<https://www.ncbi.nlm.nih.gov/sra?term=SRP067106>.
- 266 17. Zore, Gajanan 2017: Untitled Item. figshare. Dataset, Whole genome sequence of
267 *Candida albicans* ATCC10231. <https://doi.org/10.6084/m9.figshare.5349937.v1>.
- 268 18. Zimin AV, Marçais G, Puiu D, Roberts M, Salzberg SL, Yorke JA. 2013. The
269 MaSuRCA genome assembler. Bioinformatics 29:2669-2677.
- 270 19. Luo R(1), Liu B, Xie Y, Li Z, Huang W, Yuan J, He G, Chen Y, Pan Q, Liu Y,
271 Tang J, Wu G, Zhang H, Shi Y, Liu Y, Yu C, Wang B, Lu Y, Han C, Cheung DW,
272 Yiu SM, Peng S, Xiaoqian Z, Liu G, Liao X, Li Y, Yang H, Wang J, Lam TW,

273 Wang J. 2012. SOAPdenovo2: an empirically improved memory-efficient short
274 read de novo assembler. *GigaScience* 1:18.

275 20. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M., Kulikov AS, Lesin
276 VM, Nikolenko SI, Pham S, Prjibelski. AD, Pyshkin AV, Sirotkin AV, Vyahhi N,
277 Tesler G, Alekseyev MA, Pevzner PA.. 2012. SPAdes: A New Genome Assembly
278 Algorithm and Its Applications to Single-Cell Sequencing. *J Comp Biol*
279 19(5):455-477.

280 21. Stanke M, Steinkamp R, Waack R, Morgenstern B. 2004. AUGUSTUS: a web
281 server for gene finding in eukaryotes. *Nucleic Acids Res.* 32:W309–W312.

282 22. Gish W, States DJ. 1993. Identification of protein coding regions by database
283 similarity search. *Nature Genet.* 3, 266-272.

284 23. Skrzypek MS, Binkley J, Binkley G, Miyasato SR, Simison M, Sherlock G. 2017.
285 The *Candida* Genome Database (CGD): incorporation of Assembly 22, systematic
286 identifiers and visualization of high-throughput sequencing data. *Nucleic Acids*
287 *Res.* 45:D592-D596.

288 24. Li HA 2011. Statistical framework for SNP calling, mutation discovery, associa-
289 tion mapping and population genetical parameter estimation from sequencing data.
290 *Bioinformatics* 27:2987–93.

291 25. Cingolani P, Platts A, Wang le L, Coon M, Nguyen T, Wang L, Land SJ, Lu X,
292 Ruden DM. 2012. A program for annotating and predicting the effects of single
293 nucleotide polymorphisms, SnpEff: SNPs in the genome of *Drosophila melano-*
294 *gaster* strain w1118; iso-2; iso-3. *Fly (Austin).* 6(2): 80-92.

295 26. Souza ACR, Fuchs BB, Pinhati HMS, Siqueira RA, Hagen F, Meis JF, Mylonakis
296 E, Colombo AL. 2015. *Candida parapsilosis* resistance to fluconazole: molecular
297 mechanisms and *in vivo* impact in infected *Galleria mellonella* larvae. *Antimicrob*
298 *Agents Chemother* 59:6581-7.

- 299 27. Calderon J, Zavrel M, Ragni E, Fonzi WA, Rupp S, Popolo L. 2010. PHR1, a pH-
300 regulated gene of *Candida albicans* encoding a glucan-remodelling enzyme, is re-
301 quired for adhesion and invasion. Microbiology 156:2484-94.
- 302 28. Davis D. 2003. Adaptation to environmental pH in *Candida albicans* and its rela-
303 tion to pathogenesis. Curr Genet 44:1-7.
- 304 29. Raja JG, Davis DA. 2012. The beta-arrestin-like protein Rim8 is
305 hyperphosphorylated and complexes with Rim21 and Rim101 to promote adapta-
306 tion to neutral-alkaline pH. Eukaryot Cell 11:683-93.
- 307 30. Warena AJ, Kauffman S, Sherrill TP, Becker JM, Konopka JB. 2003. *Candida*
308 *albicans* septin mutants are defective for invasive growth and virulence. Infect
309 Immun 71:4045-51.
- 310 31. Hall RA, Bates S, Lenardon MD, Maccallum DM, Wagener J, Lowman DW,
311 Kruppa MD, Williams DL, Odds FC, Brown AJ, Gow NA. 2013. The Mnn2
312 mannosyltransferase family modulates mannoprotein fibril length, immune recog-
313 nition and virulence of *Candida albicans*. PLoS Pathog 9:e1003276.
- 314 32. Bates S, Hughes HB, Munro CA, Thomas WP, MacCallum DM, Bertram G, Atri-
315 A, Ferguson MA, Brown AJ, Odds FC, Gow NA. 2006. Outer chain N-glycans are
316 required for cell wall integrity and virulence of *Candida albicans*. J Biol Chem
317 281(1):90-8.
- 318 33. Castillo L, Calvo E, Martínez AI, Ruiz -Herrera J, Valentín E, Lopez JA,
319 Sentandreu R. 2008. A study of the *Candida albicans* cell wall proteome. Prote-
320 omics 8(18):3871-81.
- 321 34. Trunk K, Gendron P, Nantel A, Lemieux S, Roemer T, Raymond M. 2009. Deple-
322 tion of the cullin Cdc53p induces morphogenetic changes in *Candida albicans*.
323 Eukaryot Cell 8:756-67.

35. Andes D, Lepak A, Pitula A, Marchillo K, Clark J. 2005. A simple approach for estimating gene expression in *Candida albicans* directly from a systemic infection site. *J Infect Dis* 192, 893-900.
36. McNeil JB, Flynn J, Tsao N, Monschau N, Stahmann K, Haynes RH, McIntosh EM, Pearlman RE. 2000. Glycine metabolism in *Candida albicans*: characterization of the serine hydroxymethyltransferase (SHM1, SHM2) and threonine aldolase (GLY1) genes. *Yeast* 16,167-75.
37. Jong AY, Chen SH, Stins MF, Kim KS, Tuan TL, Huang SH. 2003. Binding of *Candida albicans* enolase to plasmin(ogen) results in enhanced invasion of human brain microvascular endothelial cells. *J Med Microbiol* 52:615-22.
38. Raman SB, Nguyen MH, Zhang Z, Cheng S, Jia HY, Weisner N, Iczkowski K, Clancy CJ. 2006. *Candida albicans* SET1 encodes a histone 3 lysine 4 methyltransferase that contributes to the pathogenesis of invasive candidiasis. *Mol Microbiol* 60:697-709.
39. Chang P, Fan X, Chen J. 2015. Function and sub-cellular localization of Gcn5, a histone acetyltransferase In Calderone RA, Clancy CJ (ed) *Candida and Candidiasis*, 2nd edn (ASM Press, Washington, DC 2011).

Figure Legends

349

350 Figure 1: BLASTX E-value distribution and BLASTX similarity score distribution

351 (a) Around 93% of the CDS found using BLASTX have a confidence level of at least
 352 1e-50, which indicates high protein conservation. (b) 99.80% of the predicted CDS
 353 found using BLASTX have a similarity of more than 60% at the protein level with the
 354 existing proteins at NCBI database.

355

356
357
358
359

360 **Figure 2. Organism annotation**

361 Top 15 BLASTX organism hits with corresponding ORF count. Among the total 32
362 organisms found in annotation, the top hit was found to be *C. albicans*.

363
364
365
366
367
368
369

Figure 3: GO annotation according to biological processes, molecular function and cellular component.

The top 15 gene ontology (GO) terms for predicted CDS identified in (a) biological process, (b) molecular function and (c) cellular component category.

396

397

398

399

400

401

402

403

404

405

406

407

408

409

410

411

412

413

414

415

416

417

420 **Figure 4. Mapping quality (MAPQ) and Insert size distribution of the aligned**

421

422 **reads with *C. albicans* SC531 and *C. albicans* WO1).**

423

(4a) 14. 2 million (~88%) of the total pre-processed reads were mapped to the *C.*

424 *albicans* strain SC5314 (RG 1) with an average mapping quality of 45.24 and (4b)

425 11.7 million (~72%) of the total pre-processed reads were mapped to the *C. albicans*

426 strain WO1 (RG 2) with an average mapping quality of 54.31. The insert size distribu-

427 tion of the reads with respect to (4c) *C. albicans* strain SC5314 (RG 1) and (4d) *C.*

428 *albicans* strain WO1 (RG 2). The X-axis (of c,d), denotes the insert size (in bp) and

429 Y-axis denotes the number of passed reads. The insert size distribution shows a peak

430 at 300bp.

Table 2. Genome assembly using different tools

Assembler	Contig length (bp)			N50	Contigs	
	Minimum	Maximum	Mean		Number	Total length (bp)
Spades	80	110945	2421.19	8971	6339	15347969
Masurca	300	231110	7565.45	28273	2262	17113050
SOAP	100	187314	667.44	4567	28492	19016774

432

433

434

Table 3. Quality score distribution

435

436

Score	SNP	Indel	SNP	Indel
50-60	2024	198	2297	528
60-70	1926	181	2762	432
70-80	1960	158	3105	392
80-90	1945	128	3340	339
90-100	1984	139	3645	358
>=100	22964	2102	69941	7488

437

438

Table 4. Annotation of variants

439

REGION	SNP	INDEL	SNP	INDEL
Intergenic	17,052	2,108	43,979	7,166
InsideGene	15,751	798	41,111	2,371
Exonic	15,751	798	40,841	2,343
Coding Region	15,505	789	40,805	2,336
NonCoding Gene	47	3	30	6
PtCodingGene ncRNA	199	6	6	1
Intronic	NA	NA	270	28
5' splice site	NA	NA	3	0
Other	NA	NA	267	28

440

441

442

443

444

Table 5. Variant class summary

Variants	<i>C. albicans</i> SC5314	<i>C. albicans</i> WO1
Frame Shift	173	399
In Frame	744	2,017
Missense	6,768	14,74
Nonsense	59	80
Silent	10,024	26,358
Start loss	23	18
Stop loss	27	29

Table 6. Identified variants

Variants	<i>C. albicans</i> SC5314	<i>C. albicans</i> WO1
Total Variants	72,036	1,11,011
Passed Variants	35,709	94,627
Total SNPs	32803 (91.86%)	85090 (89.92%)
Total inDels	2906 (8.14%)	9537 (10.08%)
Total Homozygous SNP	21606 (65.86%)	41703 (49.01%)
Total Heterozygous SNP	11197 (34.13%)	43387 (50.99%)
Total transition type SNP	23324 (71.1%)	62049 (72.92%)
Total Transversion type snp	9479 (28.89%)	23041 (27.08%)
Ts/Tv	2.46	2.69

Table 7. Read depth distribution of the identified variants with *C. albicans* SC5314 and WO1.

Read Depth	<i>C. albicans</i> SC5314		<i>C. albicans</i> WO1	
	Total SNPs	Total INDELS	Total SNPs	Total INDELS
0-5	0	0	0	0
5-10	0	0	0	0
10-15	2148	137	712	162
15-20	2894	344	1700	453
20-25	2937	360	2680	720
25-30	2719	339	3774	824
30-50	9072	971	23302	3547
>=50	13033	755	52922	3831

464 **Table 8. Variants associated with fluconazole resistance, pH response and noninvasive growth in**
465 ***Candida albicans* ATCC10231.**

Gene	Function	SNP-Indels
Fluconazole resistance		
ERG6	Delta(24)-sterol C-methyltransferase.	3
ERG11	Lanosterol 14-alpha-demethylase.	3
CDR3	Transporter of the Pdr/Cdr family of the ATP-binding cassette superfamily	4
POR1	Mitochondrial outer membrane porin.	3
CDR4	Putative ABC transporter superfamily.	1
NDT80	Meiosis-specific transcription factor; activator of CDR1 induction.	9
HOG1	MAP kinase of osmotic-, heavy metal-, and core stress response.	4
pH response		
VPS36	ESCRT II protein sorting complex subunit, involved in RIM8 processing	32
RIM21	Plasma membrane pH-sensor involved in the Rim101 pH response pathway.	1
RIM9	Protein required for alkaline pH response via the Rim101 signaling pathway.	36-1
CSR1	Putative phosphatidylinositol transfer protein.	16
PHR1	Cell surface glycosidase.	7
RIM13	Protease, Mediate activation of Rim101 via C-terminal cleavage.	7
vps28	ESCRT I protein sorting complex subunit; involved in activation of Rim101.	4
SIT4	Serine/threonine protein phosphatase catalytic subunit.	7
CCC1/4	Manganese transporter; required for normal filamentous growth	4
Invasive growth		
MNN2	Alpha-1,2-mannosyltransferase.	2
OCH1	Alpha-1,6-mannosyltransferase, mouse intravenous infection	1
PHR1	Cell surface glycosidase, involved in systemic infection.	7
CDC10	Septin, role in virulence and kidney tissue invasion in mouse infection.	4
TMA1	Cell wall protein,	6
PGA30	GPI-anchored protein of cell wall.	4
CDC53	Cullin, a scaffold subunit of the SCF ubiquitin-ligase complexes;	3
RSP5	Putative NEDD4 family E3 ubiquitin ligase, induced during infection of murine kidney	11
SHM2	Cytoplasmic serine hydroxymethyltransferase	13
ENO1	Enolase; glycolysis and gluconeogenesis	7
SET1	H3k4	4
POB3	Protein involved in chromatin assembly and disassembly.	9
HHT2	Putative histone H3	5
GCN5	Histone acetyltransferase.	6
HDA1	Histone deacetylase; inducer of filamentation.	14
CCC1	Manganese transporter; required for normal filamentous growth	4
HOG1	MAP kinase of osmotic-, heavy metal-, and core stress response.	4
RCK2	Predicted MAP kinase-activated protein kinase.	5
RIM13	mediate activation of Rim101 via C-terminal cleavage.	7
NDT80	Meiosis-specific transcription factor.	9
DAM1	microtubule binding.	4
NAM2	Mitochondrial leucyl-tRNA synthetase.	47
TIM50	mitochondrial transport.	5
NCE102	Non classical protein export protein; localized to plasma membrane	4
GPR1	Plasma membrane G-protein-coupled receptor of the cAMP-PKA pathway.	20-1
FET3	Putative copper ferroxidase involved in iron uptake.	18
MOB1	Putative mitotic exit network component; periodic mRNA expression	4
RRP6	Putative nuclear exosome exonuclease component.	14
ENP2	Putative nucleolar protein.	15-1
RPC40	Putative RNA polymerase.	5
MID1	Putative stretch-activated Ca ²⁺ channel of the high affinity calcium uptake system.	4
SIT4	Serine/threonine protein phosphatase catalytic subunit, involved in alkaline pH sensing.	7
SMC5	Smc5p, which is involved in DNA repair.	42
BMH1	Sole 14-3-3 protein in <i>C. albicans</i> .	1
STP4	C2H2 transcription factor.	3
PZF1	C2H2 transcription factor.	9
ASH1	GATA-like transcription factor.	4-2
CAT8	Zn(II)2Cys6 transcription factor.	12
WOR2	Zn(II)2Cys6 transcription factor. w/o regulator	5-1
CTA7	Zn(II)2Cys6 transcription factor.	9

466