

Minireview

A *Candida*-based view of fungal sex and pathogenesis

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Abstract

Analysis of the genomes of multiple *Candida* species reveals shared traits associated with pathogenesis, but also striking differences in sexual reproduction.

Yeasts belonging to the *Candida* group of species are opportunistic pathogens responsible for both debilitating mucosal infections (such as oral thrush and vaginitis) as well as potentially life-threatening systemic infections in humans. These fungi represent a diverse collection of hemiascomycetes, although the majority of *Candida* species are grouped together in a single clade, termed the *Candida* clade. In a recent study, Butler *et al.* [1] have sequenced the genomes of six species from the *Candida* clade and compared them with genomes from non-pathogenic yeasts. They show that gene families associated with pathogenesis have expanded in species from the *Candida* clade, suggesting that this expansion coevolved with the ability to cause infection of the host. In addition, the authors demonstrate a surprising divergence in the mechanisms regulating mating and meiosis in this clade. These studies provide an exciting entry point for comparative analysis of *Candida* species; future studies will compare and contrast how these species acquired pathogenicity as well as potentially novel modes of sexual reproduction in these yeasts.

***Candida* genomes**

The six species sequenced from the *Candida* clade included an isolate of *Candida albicans*, the most frequently isolated species from humans, as well as the closely related pathogens *C. parapsilosis* and *C. tropicalis* (Figure 1). A fourth species, *Lodderomyces elongisporus*, was originally thought to be non-pathogenic but has recently been isolated from multiple bloodstream infections [2]. Each of these four species exists primarily in the diploid state. In addition, the genomes of two emerging haploid pathogens, *C. guilliermondii* and *C. lusitaniae*, were analyzed. These sequences were compared to the genome of the non-pathogenic *Debaryomyces hansenii*, a halotolerant yeast found in the environment, as well as multiple species from the *Saccharomyces* clade.

The *Candida* genomes showed a broad distribution of sizes, ranging from 10.6 to 15.5 megabases (Mb), although the predicted number of protein-coding genes was very similar in each (5,733 to 6,318). This apparent discrepancy was attributed to variability in intergenic spacing among species. General chromosomal configurations were similar (each species contained between six and nine chromosomes), although synteny has been disrupted by intra-chromosomal rearrangements since these species diverged. Interestingly, only *C. albicans* and *C. tropicalis* contained the major repeat sequence (MRS) elements. These are important sites for genetic recombination in *C. albicans* as they promote chromosome translocations and increase genomic diversity [3]. The discovery of MRS elements in *C. tropicalis* suggests that these repeats play a similar role in karyotypic variation in these strains [4], although the contribution of these changes to pathogenesis is not known.

A characteristic feature of the diploid *C. albicans* genome is significant sequence divergence between chromosome homologs. Thus, one single nucleotide polymorphism (SNP) was found every 330 bp in the most commonly studied strain, SC5314, and similarly, one SNP occurred every 390 bp in the clinical WO-1 isolate. The other diploid *Candida* clade species also exhibit SNPs, ranging from one SNP every 222 bases in *L. elongisporus* to one SNP per 15,553 bases in *C. parapsilosis*. Thus, heterozygosity is common in the diploid *Candida* species, but the extent of polymorphism varies greatly between them. Three species also show long stretches of homozygosity, and this was most dramatic in the *C. albicans* WO-1 strain, with 30% of the genome lacking SNPs. It is unclear if these homogenous regions are due to break-induced replication or are the result of progress through a parasexual or sexual cycle.

Evolution of pathogenicity

Butler *et al.* [1] identified a number of expanded gene families in the *Candida* clade that are associated with pathogenesis. Out of 9,209 gene families, 21 were enriched in the pathogenic species compared to the non-pathogens. These included three families of cell-wall proteins, most of which contain putative carboxy-terminal glycosyl phosphatidylinositol (GPI) anchors that mediate covalent

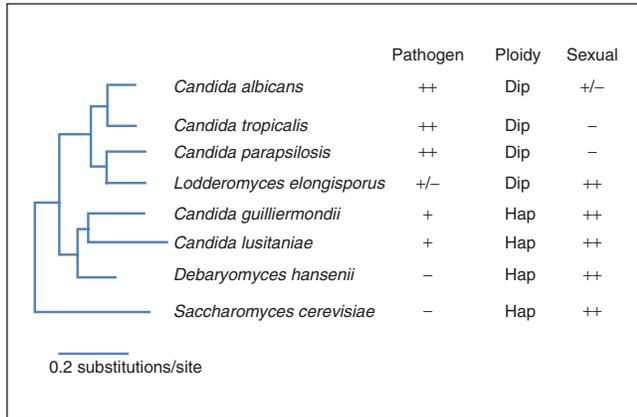


Figure 1

Phylogenetic tree of the *Candida* clade of species, together with the related hemiascomycete *S. cerevisiae*. The six species sequenced in the Butler *et al.* study [1] were *C. albicans*, *C. tropicalis*, *C. parapsilosis*, *L. elongisporus*, *C. guilliermondii*, and *C. lusitanae*. These sequences were compared to those of non-pathogenic yeasts, including *D. hansenii* and species from the *S. cerevisiae* clade. Note that *L. elongisporus* has now been isolated from bloodstream infections (a rare pathogen), and that *C. albicans* undergoes efficient mating but a conventional meiosis has not been identified (parasexual reproduction). Figure adapted from the web site of the Broad Institute. Dip, diploid genome; Hap, haploid genome.

attachment to the fungal cell wall. The *ALS* gene family encodes eight cell-wall proteins that have been studied in detail in *C. albicans*. These proteins (particularly Als1 and Als3) promote adhesion to epithelial cells, although other functions for this family have emerged, including a role in biofilm formation [5-7]. Similarly, the family of Hyr/Iff proteins was enriched in the *Candida* clade. At least one member of this family, *IFF11*, was recently shown to lack a GPI anchor and is secreted in *C. albicans*. Loss of *IFF11* results in defective cell-wall structure and reduced virulence [8]. The third family of cell-wall proteins enriched in *Candida* includes members related to Pga30. Less is known about this family of proteins, although several members were identified in the *C. albicans* cell-wall proteome [9]. All three families of cell-wall proteins show high mutation rates, suggesting that these are rapidly evolving factors that provide *Candida* species with a selective advantage for invasion and infection of the mammalian host.

Interestingly, the family of Hwp1-related cell-wall proteins is limited to only a subset of *Candida* species. In *C. albicans*, Hwp1 plays an important role in biofilm formation and the covalent attachment of hyphae to host epithelial cells via mammalian transglutaminases [7,10]. However, genes encoding Hwp1-like proteins were found only in the diploid *Candida* species. In addition, only the *C. albicans* Hwp1 protein contained the amino-terminal

transglutaminase-substrate domain [1]. Comparative genome analyses can therefore provide revealing insights into why *Candida* species differ in their ability to colonize and infect the human body. Another striking example of a species-specific gene set was that encoding Fgr38-like family members, with 33 genes present in *C. albicans* and only one in all the other *Candida* species combined. Fgr38 was originally identified in a screen for factors that affect hyphal growth [11]. Such large-scale amplification of a gene family in *C. albicans* suggests that it might play an important, but as yet largely undefined, role in promoting the fitness of this species.

Expansion of multiple gene families associated with extracellular enzymes and transmembrane transporters was also noted in *Candida* [1]. These include oligopeptide transporters, amino-acid permeases, lipases, superoxide dismutases, ferric reductases and GPI-anchored yapsin proteases. Extracellular hydrolytic enzymes (for example, secreted aspartyl proteases, lipases and phospholipases) are known to be necessary for virulence in *Candida*, and are involved in nutrient acquisition, tissue invasion and evasion of the immune response [12,13]. The selective expansion of these gene families in *Candida* again attests to the importance of these genes in colonization and pathogenesis.

It is also revealing that *C. glabrata*, a prevalent pathogen that is closely related to *Saccharomyces cerevisiae*, did not show the same expansion of genes as the other *Candida* species. Thus, while *C. albicans* had 161 representatives in the 21 gene families enriched in pathogenic *Candida*, *C. glabrata* contained only three genes from these families [1]. These observations demonstrate that *C. glabrata* has evolved alternative mechanisms from most of the other *Candida* species for infection of the host. This view is also consistent with studies on *C. glabrata* that have established that traits associated with virulence in *C. albicans* (for example, hyphal growth and secreted proteinase activity) are not utilized by *C. glabrata* [12]. Despite these fundamental differences, *C. glabrata* and *C. albicans* do share general features necessary for virulence, including phenotypic plasticity and expanded numbers of host adhesins [12].

Sexual identity, mating and meiosis

Mating in the hemiascomycete yeast is regulated by transcription factors encoded at the mating-type (*MAT*) loci. These are master regulators of cell identity, defining the programs of sexual differentiation in these species. The two idiomorphs of the locus are *MATa* and *MAT α* , which direct cells to mate as **a** or α cell types, respectively. Interestingly, although these loci have been shown to regulate sexual identity in both *S. cerevisiae* and *C. albicans* [14,15], the transcriptional circuitry by which they do so has diverged between these species [16].

Analysis of the *MTL* (mating-type-like) locus in different *Candida* species revealed a surprising diversity in *MTL* organization [1]. Although synteny surrounding the *MTL* locus has been largely conserved, the presence of the master transcriptional regulators themselves is highly variable. In *C. albicans*, which appears to have retained the ancestral *MTL* configuration, *MTLa* encodes **a1** and **a2** transcription factors, and expression of **a2** is required for the cell to mate as an **a**-type cell. Conversely, *MTL α* includes $\alpha 1$ and $\alpha 2$, and expression of $\alpha 1$ is necessary for α mating-type characteristics [16]. In both *S. cerevisiae* and *C. albicans*, **a1** forms a heterodimer with $\alpha 2$ that represses expression of mating genes, while in *S. cerevisiae* **a1**/ $\alpha 2$ also facilitates entry of diploid cells into meiosis [14,16]. Curiously, the *MTL $\alpha 2$* gene was found to be missing from all three haploid *Candida* species [1]. This was not associated with an inability to undergo sexual reproduction; Reedy *et al.* [17] recently confirmed that *C. lusitaniae* undergoes efficient mating and meiosis even in the absence of $\alpha 2$, and *C. guilliermondii* and *D. hansenii* also have complete sexual cycles ([18] and references therein). Rather, these experiments indicate that the transcriptional regulation of meiosis has been rewired in these species, with another α -specific factor (perhaps $\alpha 1$) taking the place of $\alpha 2$ [17].

Other differences at the *MTL* locus were noted, with the most striking occurring in *L. elongisporus*, where all four mating-type regulators (**a1**, **a2**, $\alpha 1$ and $\alpha 2$) have been lost. This fungus is thought to be homothallic (self-mating) [2] and may be the first ascomycete identified to undergo sexual reproduction in the absence of either *MTLa* or *MTL α* [1]. *D. hansenii* is also homothallic, and in this case both *MTL* loci have become fused into a single locus. Similar fusion of *MTL* loci has been described in other homothallic ascomycetes, suggesting that this is a common mechanism by which homothallism can evolve from a heterothallic (cross-mating) ancestor ([18] and references therein).

The meiotic program has undergone considerable changes in the *Candida* lineage. The transcription factor gene *IME1*, the major regulator of meiosis in *S. cerevisiae*, is absent, yet the three established sexual species (*C. lusitaniae*, *C. guilliermondii* and *D. hansenii*) can undergo meiosis without it [1,17]. *C. albicans* strains undergo efficient mating between **a** and α forms [14,15], yet completion of the sexual cycle occurs by a parasexual mechanism of random chromosome loss rather than conventional meiosis ([19] and references therein). On the basis of genomic evidence, *C. albicans* either has a cryptic meiotic program that has yet to be identified, or conserved 'meiosis-specific' genes have been reprogrammed to function in the parasexual cycle [1]. *C. guilliermondii* and *C. lusitaniae* have also lost multiple components of the meiotic machinery. These include parts of the

synaptonemal complex (Hop1, Red1, Mek1, Zip1 and Zip2), together with the Dmc1-dependent pathway of meiotic recombination, and the crossover-formation pathway mediated by the MutS homologs Msh4/Msh5. These findings indicate that meiosis in *Candida* is fundamentally distinct from that in the model hemiascomycete, *S. cerevisiae*, in terms of both its regulation and its molecular apparatus. At least the role of Spo11 in directing DNA double-strand breaks appears to be conserved in *Candida*, as this protein mediates meiotic recombination in *C. lusitaniae* [17] as well as parasexual recombination in *C. albicans* [19].

Finally, two *Candida* species, *C. parapsilosis* and *C. tropicalis*, were found to contain intact pheromone signaling transduction pathways by genomic analysis but have never been observed to mate ([18] and references therein). Either these species recently lost the ability to undergo sexual reproduction (as evidenced by mutation of *MTLa1* in *C. parapsilosis* [20]) or cryptic mating pathways have yet to be uncovered in these species.

The wealth of information provided by Butler *et al.* [1] has opened the door for comparative studies to further illuminate *Candida* biology. Already, multiple traits associated with *Candida* pathogenesis have been inferred, including expansion of gene families associated with colonization and infection, as well as species-specific features that make each *Candida* unique. In the latter case, it is particularly intriguing that mechanisms of mating and sexual reproduction have rapidly diverged between *Candida* species. Future studies will no doubt address how these differences evolved and what role they play in the lifestyles of these opportunistic yeast.

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