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Microbe Profile: *Candida glabrata* – a master of deception

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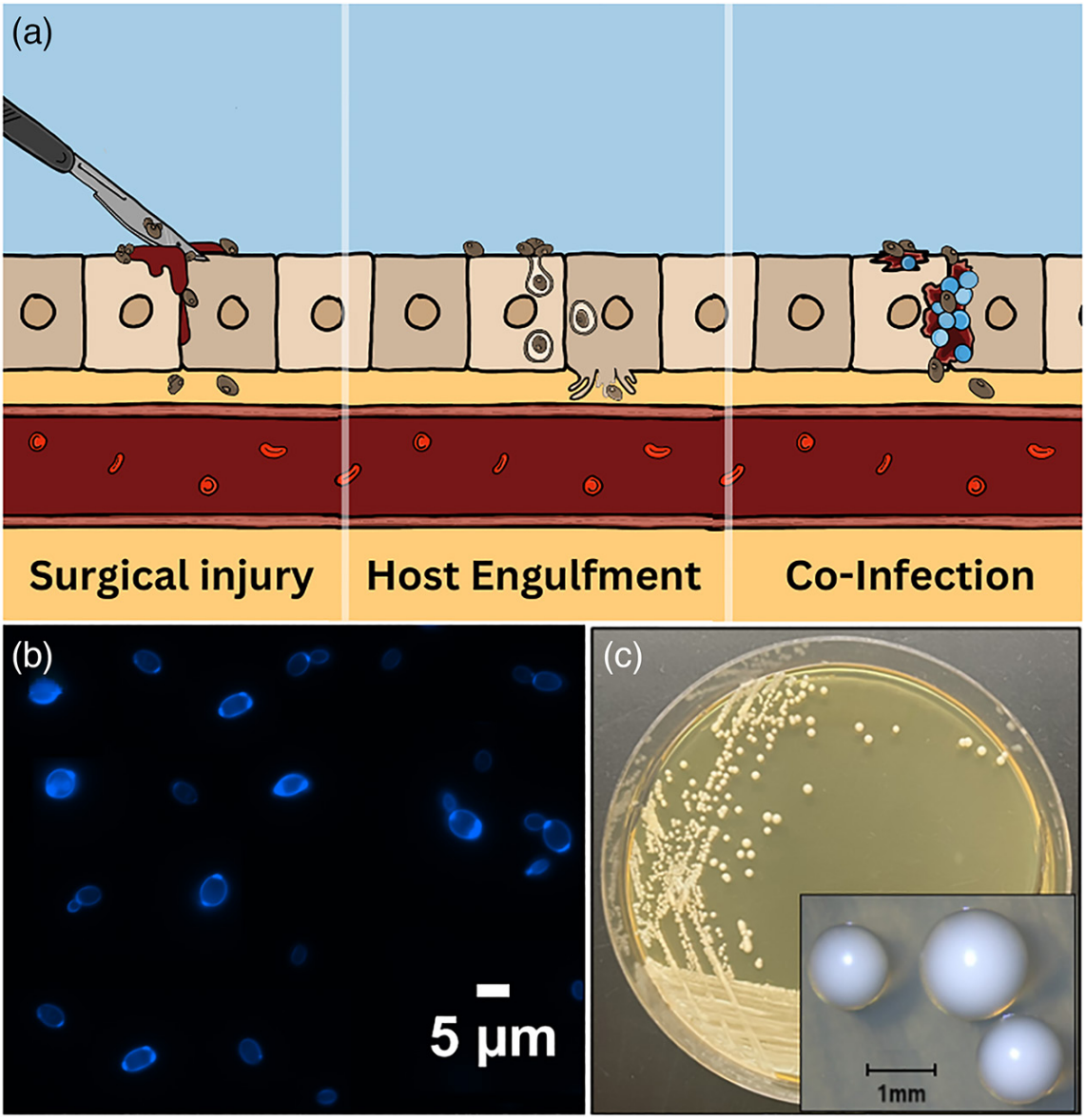

Graphical abstract

Candida glabrata, an opportunistic fungal pathogen. (a) Unable to form hyphae, relies on tissue disruption to invade and disseminate host. (b) Calcofluor-white cells, 60X objective. (c) Colony morphology on YPD.

Abstract

Candida glabrata is a fungal microbe associated with multiple vertebrate microbiomes and their terrestrial environments. In humans, the species has emerged as an opportunistic pathogen that now ranks as the second-leading cause of candidiasis in Europe and North America (Beardsley *et al*. *Med Mycol* 2024, 62). People at highest risk of infection include the elderly, immunocompromised individuals and/or long-term residents of hospital and assisted-living facilities. *C. glabrata* is intrinsically drug-resistant, metabolically versatile and able to avoid detection by the immune system. Analyses of its 12.3 Mb genome indicate a stable pangenome Marcet-Houben *et al*. (*BMC Biol* 2022, 20) and phylogenetic affinity with *Saccharomyces cerevisiae*. Recent phylogenetic analyses suggest reclassifying *C. glabrata* as *Nakaseomyces glabratus* Lakashima and Sugita (*Med Mycol J* 2022, 63: 119-132).

TAXONOMY

Candida glabrata was first isolated from human stool by Anderson (1917), who designated this isolate as *Cryptococcus glabratus* [1]. When pseudohyphae formation was found to be an unreliable taxonomic character, the organism was reclassified as *Torulopsis glabrat*a [2]. It is currently classified as follows: domain: Eukaryota, kingdom: *Fungi*, subkingdom: *Dikarya*, phlyum: *Ascomycota*, subphylum: *Saccharomycotina*, class: *Saccharomycetes*, order: *Saccharomycetales*, family: *Saccharomycetaceae*, genus: *Nakaseomyces*, clade: *Nakaseomyces/Candida* and species: *glabratus*/*glabrata*. Reclassification to *Nakaseomyces glabratus* has been proposed, based on expanded molecular and phylogenetic analyses [3].

PROPERTIES

C. glabrata is a haploid yeast averaging 3 µM in length that grows optimally at 37 °C and up to 42 °C. A facultative anaerobe, the species is auxotrophic for thiamine, pyridoxine and nicotinic acid [4]. As mating has never been observed, the organism has long been considered asexual [5]. However, recent genomic analyses provide evidence for mating-mediated recombination [6]. *C. glabrata* resides as a commensal on the epithelium of healthy individuals but can become pathogenic in those with a weakened immune system. As *C. glabrata* does not form pseudohyphae, it requires tissue barrier disruption to enter the bloodstream, where it can disseminate and evade the host immune system by invading macrophage.

GENOME AND EVOLUTION

The first complete genome sequence of *C. glabrata* was published in 2004; a revised assembly was released in 2020 [7]. The 12.3 Mb genome harbours 5272 predicted ORFs, of which 571 ORFs (10.83%) have been experimentally verified. Lengths of *C. glabrata*'s 13 nuclear chromosomes range between 512 655 and 1 528 264 bp. The nuclear genome is dynamic as evidenced by extensive chromosomal length polymorphism due to translocations and tandem gene repeats. The *C. glabrata* mitochondrion encloses a 20 kb circular genome consisting of 11 ORFs. The most frequently studied laboratory strains are CBS138 (ATCC 2001) and BG2. CBS138 was isolated from human faeces [1] and was the first to be fully sequenced. BG2 was derived from the parental strain 'B', which was a vaginitis, fluconazole-resistant isolate [8].

C. glabrata is closely related to the non-pathogenic yeast, *Saccharomyces cerevisiae*. Both species belong to the whole-genome duplication (WGD) group, and gene order along their respective chromosomes is largely conserved [9]. A noteworthy difference between the two species is the expansion of adhesion genes in *C. glabrata* [10]; these are thought to be important virulence factors, as they enable the organism to adhere to different host substrates and facilitate biofilm development [11].

C. glabrata exhibits remarkable genomic plasticity that may play a role in antifungal resistance and pathogenicity. For example, sub-telomeric adhesin genes vary both in copy number and in DNA sequence among different *C. glabrata* strains [6, 12]. Independent isolates may exhibit different karyotypes, inviting speculation that large-scale rearrangements may facilitate adaptation to novel environments. Analysis of sequential isolates among patients undergoing antifungal therapy has shown that chromosomal

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Keywords: *Candida glabrata*; candidiasis; opportunistic pathogen; yeast.
Abbreviations: GPI, glycosylphosphatidylinositol; PAMPs, pathogen-associated molecular patterns; PDR1, pleiotropic drug resistance 1; SCVs, small-colony variants; SWI/SWNF, sucrose non-fermentable ATP-dependent chromatin remodeling complex subfamily; WGD, whole-genome duplication.

rearrangements occur alongside the evolution of drug resistance. Although a causal relationship between these events remains uncertain, genome plasticity has the potential to contribute to evolutionary adaptation [13].

PHYLOGENY

Candida is a polyphyletic genus within the subphylum *Saccharomycotina,* which includes both CTG and non-CTG clades as well as pre- and post-WGD groups. *C. glabrata* and its sister taxa, including *Saccharomyces cerevisiae*, belong to the non-CTG clade that translates the CUG codon into leucine, similar to most eukaryotes. However, many other *Candida spp*. belong to the CTG (or CUG) clade, in which species translate CUG to serine [9]. While some have speculated that pathogenicity correlates with this coding shift, species with varying degrees of medical relevance exist within both clades [14]. In any case, differences within the *Saccharomycotina* in their genome sizes and coding rules likely played roles in protein diversification.

Multiple closely related pathogenic and nonpathogenic species exist within the *Nakaseomyces* clade, the newly proposed taxonomic home for *C. glabrata*. When examined as a group, these species could provide a valuable resource for experimental and comparative genomic studies aimed at discovering the molecular bases of virulence [5, 6].

KEY FEATURES AND DISCOVERIES

Little is known about the environmental reservoirs of *C. glabrata*, although the species has been recovered from settings as diverse as vertebrate hosts, including humans and birds [15], surfaces of flowers and leaves and water and soil [5]. In humans, *C. glabrata* can be a normal component of the epithelial microbiome of the skin, oral cavity, gastrointestinal tract and urogenital tract. Given this range of habitats, it is thought that *C. glabrata* can be found anywhere near human habitation.

C. glabrata is of particular clinical relevance because of its low susceptibility to azole drugs [12], which are clinically employed as a cost-effective, first-choice preventative and as a second-choice treatment for invasive candidiasis. As with other antibiotics, this practice has led to increased cross-resistance in *C. glabrata* to most types of azoles [16]. Additionally, *C. glabrata* exhibits the capacity to evolve secondary resistance to multiple antifungal drug classes (polyenes, echinocandins and flucytosine), especially after exposure to more than one drug [11, 12, 17]. One means by which azole resistance is achieved is via mutations in pleiotropic drug resistance 1 (*PDR1*), a transcription factor that controls the regulation of drug efflux pumps [9]. As *PDR1* now appears to play a role in many cellular responses, it may be better characterized as a sensor of overall cell stress [18]. Regulation of *PDR1* was recently shown to be controlled by the chromatin remodeler SWI/SNF (SWItch/Sucrose Non-Fermentable ATP-dependent chromatin remodeling complex subfamily) and the histone chaperone Rtt106, as the deletion of these genes sensitizes cells to antifungal drugs. Because Rtt106 and several components of the SWI/SNF complex are fungal-specific, this discovery opens up the possibility of new therapeutic targets [19].

Drug resistance in *C. glabrata* may also be associated with the emergence of petite or small-colony variants (SCVs). This phenotype has been observed in azole and echinocandin resistance studies using *in vitro*, *in vivo* and patient isolates [20–22]. With low prevalence and of uncertain fitness value, much remains to be learnt about this phenotype's clinical significance, warranting its surveillance.

C. glabrata can form biofilms, which appear to increase drug resistance and contribute to treatment failure. *C. glabrata* biofilms are compact structures composed of cells surrounded by an extracellular matrix consisting of proteins and carbohydrates. Biofilm-associated drug resistance is complex, as this matrix increases cell density, protects cells against drugs, upregulates genes encoding efflux pumps and favours the emergence of 'persisters', quasi-dormant cells that contribute to chronic infection [11].

To survive within its host, *C. glabrata* combines resistance and evasion strategies. For example, *C. glabrata* exhibits intrinsically high resistance to chemical stressors employed by the immune system such as increased osmolarity and reactive oxygen species and decreased pH [11]. Additionally, the yeast's metabolic flexibility enables it to survive in nutrient-limited host environments [2]. One key genetic component is yapsins, a family of glycosylphosphatidylinositol (GPI)-linked aspartyl proteases that have been expanded in *C. glabrata* (relative to *S. cerevisiae*). These 11 genes are upregulated in macrophage infection assays [23] and are essential for cell wall integrity, virulence, glucose homeostasis and epithelial adherence [2, 24].

C. glabrata employs immune evasion tactics that impair macrophage function and reduce inflammatory responses, enabling the yeast to survive and proliferate within immune cells. Macrophages initially locate yeast cells by identifying pathogen-associated molecular patterns. In *C. glabrata,* these include the cell wall polysaccharides chitin, α-mannan and β-glucan. Nutrients are severely limited inside macrophages, creating a hostile environment for the yeast once it has been engulfed [25]. However, once inside the macrophage's phagosome, *C. glabrata* has the capacity to impair phagosome maturation [2], disrupting acidification and lysosomal pathways that would otherwise destroy engulfed cells. Macrophages harbouring *C. glabrata* also produce less pro-inflammatory cytokines, which are important in recruiting other immune cells [26]. Once phagosome maturation has been subverted, *C. glabrata* is free to replicate within a nonacidic phagosome [25], which ultimately

leads to macrophage lysis due to high fungal loads. The orchestration of this process is not yet understood mechanistically. A *C. glabrata* mutant screen uncovered *mnn10* and *mnn11* deletions that failed to prevent vacuole acidification in an *ex vivo* assay [27], suggesting that mannosyltransferases impact protein glycosylation and secretion, which aid in neutralization. Yapsins also contribute to immune evasion [2, 28]. Deletion of *YPS1-11* results in higher immune activation, correlated with differential regulation of cell wall metabolism, and increased exposure of cell wall chitin and adhesin Epa1. Recent work mapping nucleosome placement in *C. glabrata* during macrophage engulfment [28] revealed widespread chromatin changes; these include SWI/SNF-mediated remodelling of immunogenic cell wall components, which repressed full immune recognition and response.

In summary, *C. glabrata* is a highly adaptable, intrinsically drug-resistant organism of increasing medical relevance. Ultimately, a better understanding of how individuals acquire, host and transmit *C. glabrata* is needed to reduce risks to human health posed by this formidable opportunistic pathogen.

OPEN QUESTIONS

- To what extent do environmental reservoirs of *C. glabrata* serve as sources for human colonization?
- Does *C. glabrata* undergo meiosis and mating? And if so, under what conditions?
- In human hosts, does long-term residence in one environment (skin, oral cavity, gastrointestinal and urinary tracts) influence the propensity for *C. glabrata* to disseminate and/or develop specific patterns of antifungal drug resistance?
- What diagnostic tools would provide clinicians with higher sensitivity, specificity and faster turn-around times to manage *C. glabrata* infections?
- What types of surveillance can be implemented at the national and international levels to control the spread of antifungal drug resistance in *C. glabrat*a?

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Conflicts of interest
The authors declare that there are no conflicts of interest.

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