Exploring Antifungal Drugs Produced by Brewer's Yeasts

University of Idaho
Department of Biological Sciences

Lance R. Fredericks, Cooper R. Roslund, Angela Crabtree, and Paul A. Rowley

Department of Biological Sciences, University of Idaho, Moscow, Idaho, USA.

Fungal infections kill an estimated 1.5 million people every year and affect over 1 billion. Of these infections, roughly 700,000 are cases of invasive candidiasis [1]. While antifungal drugs, such as fluconazole, have traditionally been used for treating candidosis, there has been a significant increase in drug resistant *Candida* infections. Of particular concern is one of the leading causative agents of recurrent drug-resistant vulvo-vaginal candidosis, *Candida glabrata*. Due to the high prevalence of drug resistance, infections caused by *C. glabrata* present a serious global health risk.

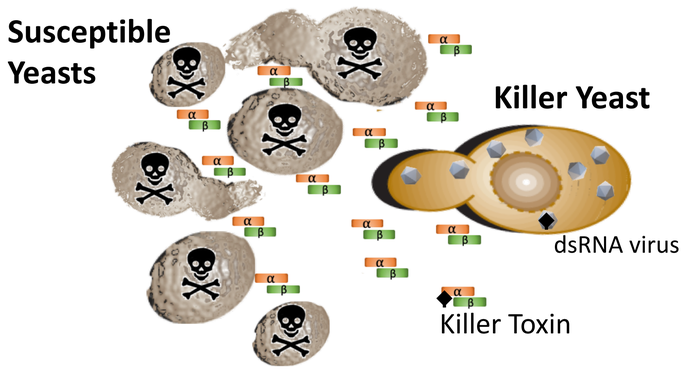


Fig 1. Killer yeasts inhibit the growth of neighboring competitor yeasts by producing killer toxins encoded by double stranded RNA satellites.

Killer Yeasts

Antifungal toxins are often produced by fungi, including *Saccharomyces cerevisiae*. Certain strains of these "killer yeasts" play host to different types of double-stranded RNA totiviruses and their associated satellites. The latter are known to encode protein toxins. These toxins provide a competitive advantage against neighboring susceptible yeasts **(Fig. 1)**. To observe the inhibitory effect of the toxin we use a "Killer Yeast Assay" which involves placing killer yeasts on an agar plate covered in a lawn of susceptible yeast cells **(Fig. 2)**.

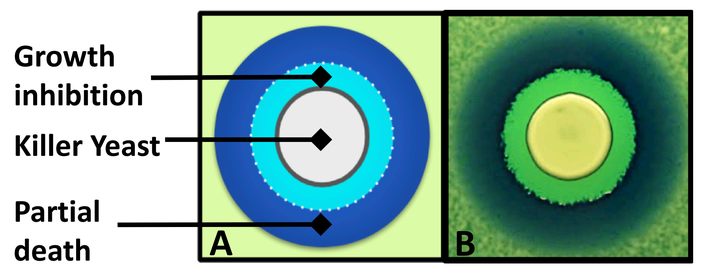


Fig. 2. Killer yeasts inhibit the growth of susceptible yeasts on solid agar. (A) Diagram of the killer phenotype exhibited by killer yeasts. (B) *Saccharomyces cerevisiae* strain BJH001 inhibiting the growth of *Candida glabrata.*

Killer Yeasts vs Pathogens

We screened a wide variety of killer yeasts against a diverse range of fungal pathogens including those from the genus *Candida, Crypotococcus, Rhodotorula,* and *Clavispora*. We determined that *C. glabrata* is more susceptible to killer toxins compared to other pathogenic yeasts **(Table 1).** We tested over 9,000 interactions between killer yeasts and various clinical and non-clinical isolates of *C. glabrata* **(Fig. 3)**. We found all drug-resistant strains of *C. glabrata* from the CDC/FDA were susceptible to killer toxins **(Fig. 4)**.

Pathogen	% of Killers that inhibit
C. glabrata	**36**
C. rugosa	19
C. saitoana	4
C. albicans	2
C. auris	2
C. lusitaniae	0
C. uzbekistanensis	0
C. railensis	0
C. carnescens	0
C. tephrensis	0
R. mucilaginosa	0

Table 1. Various fungal pathogens ranked by their susceptibility to killer toxins.

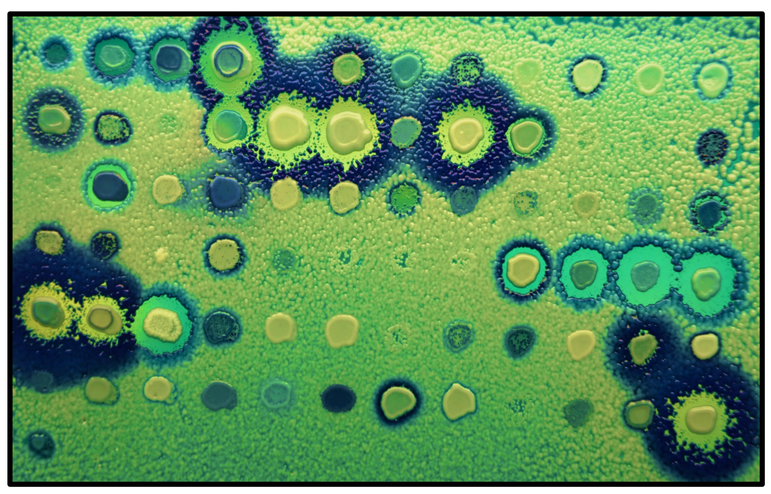


Fig. 3. A large-scale killer assay plate showing the inhibition of *C. glabrata* growth by killer yeasts.

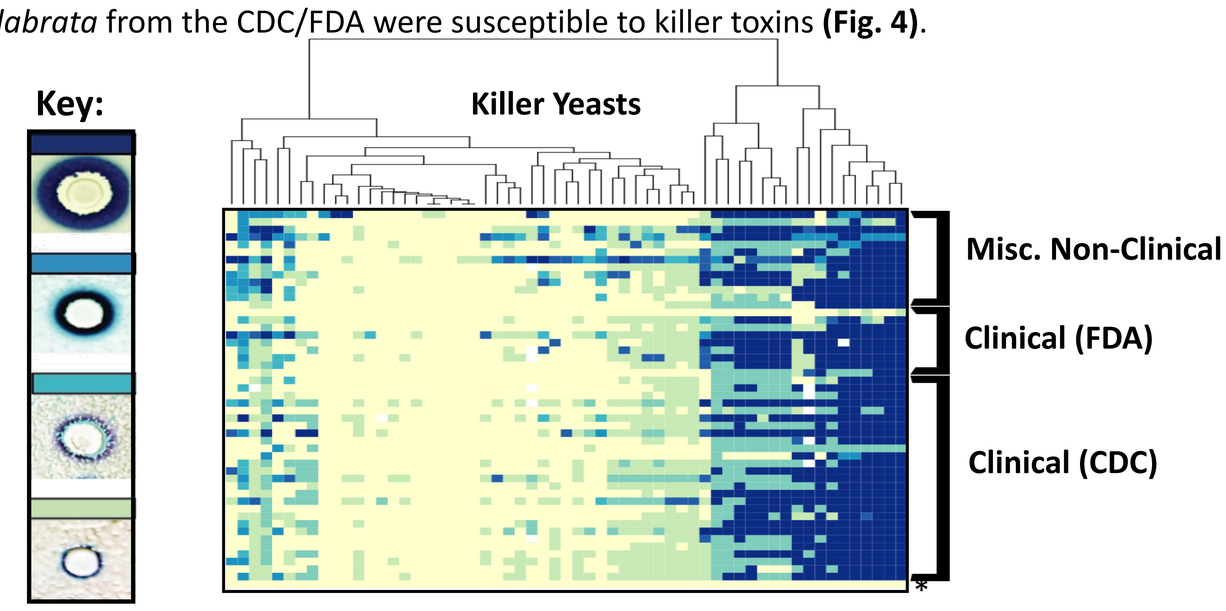
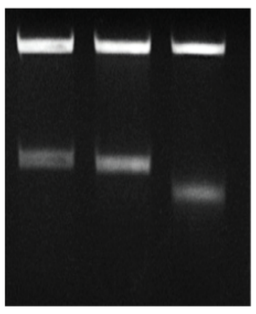


Fig. 4. A cluster diagram showing the interactions between killer yeasts and *C. glabrata* (lawn yeast). **C. albicans* (negative control)

Characterization of Killer Toxins



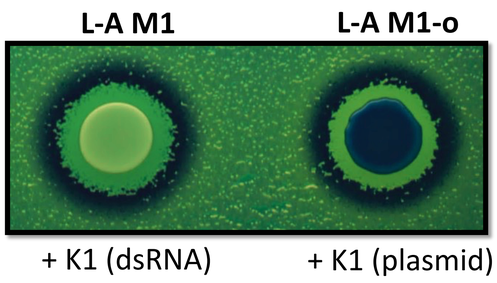


Fig 4. An agarose gel showing the dsRNA isolated from a killer yeast (top). Ectopic expression of K1 in *S. cerevisiae* can inhibit the growth of *C. glabrata* (bottom).

After finding that killer yeasts were capable of inhibiting the growth of several pathogens we extracted the dsRNA satellite elements and used Next Generation Sequencing to determine which genes coded for the the broadly inhibitory toxins. We then used an ectopic expression system to show that the toxin gene on the dsRNA satellite is responsible for the phenotype seen in the killer assays **(Fig 4.)**.

To further test our hypothesis that these toxins may be precursors to therapeutics we examined their pH range **(Fig. 5)**. While we determined they do not have significant potential for systemic infections they are very active at the average pH of vaginal mucosa (~4.5)[3].

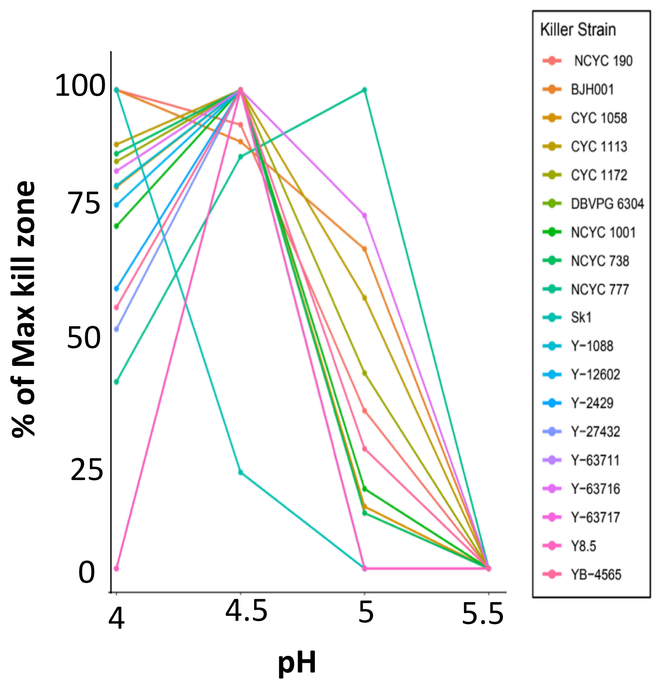


Fig. 5. The effective pH range of killer toxins.

Evolution of Killer Toxin Resistance

It is known that gene deletion can cause killer toxin resistance in *S. cerevisiae* [2]. To determine if *C. glabrata* has the potential to evolve resistance to killer toxins we challenged cultures of *C. glabrata* and *S. cerevisiae w*ith purified killer toxins.

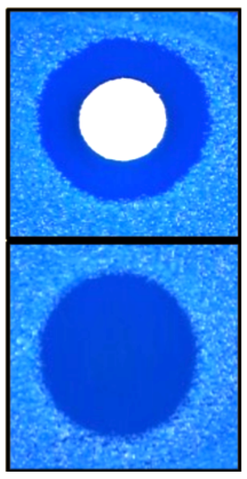


Fig. 6: A K1 killer yeast (top) with its precipitated toxin (bottom)

After recovery from exposure to killer toxin, several distinct colony morphologies were observed amongst resistant mutants **(Fig. 7)**. In particular, we observed differences in methylene blue staining, a redox indicator of cell death. Isolation of killer toxin resistant clones were used to confirm that the killer toxin resistance phenotype is stable. We have observed at least 8 different categories of resistance mutants based on their toxin susceptibility and growth rate **(Table 2)**.

Category	K1 Resistance	K2 Resistance	Growth Rate	Frequency
1	None	Partial	Fast	17%
2	None	Partial	Slow	8%
3	None	Complete	Fast	20%
4	Partial	Partial	Fast	25%
5	Partial	Complete	Slow	4%
6	Partial	Complete	Moderate	4%
7	Partial	Complete	Fast	4%
8	Complete	Complete	Fast	4%

Table 2. Categorization of resistance mutants.

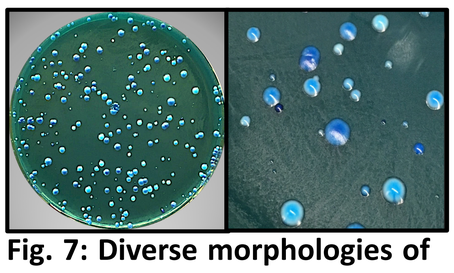


Fig. 7: Diverse morphologies of K1 killer toxin resistant mutants.

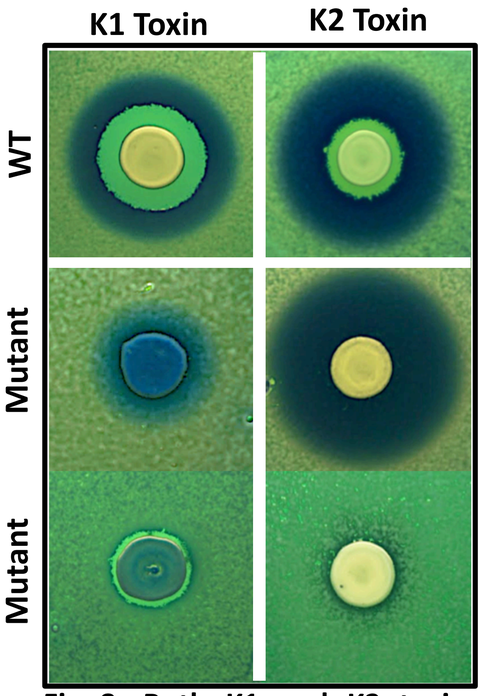


Fig 8. Both K1 and K2 toxin producing yeasts in killer assays against WT *C. glabrata* (top) and resistant mutants (bottom).

Acknowledgments

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