Antifungal Drug Sensitivity of Modified [redacted] and Unmodified [redacted]

In order to confirm that an unmodified [redacted] *S. cerevisiae* strain and [redacted] engineered strains are sensitive to clinical antifungal drugs, minimal inhibitor concentration (MIC) was measured with two antifungal drugs typically used to treat yeast infections as described in Barchiesi *et al.* (1998)[1], Amphotericin B and Fluconozole. Two additional industrial yeast strains were used as controls, [redacted] (**Table 1**).

The strains were plated on Yeast Nutrient Base (YNB) media plates with different concentrations of antifungal (Fluconazole Sigma# F8929, ≥ 98% HPLC Powder; Amphotericin B Sigma# A4888, ≈80% HPLC Powder), using cells plated on YNB with no antifungal as the control count plates. The plates were incubated at 35°C for 2 days and formed colonies were counted. MIC was defined as the antifungal concentration at which the amount of colonies on the antifungal plates compared to the control YNB (containing no antifungal) was decreased by >90%. **Table 2** shows that the engineered yeast strains [redacted] have equivalent or lower MIC to the antifungals compared to the control strains. All raw data can be found in **Table 3** and **Table 4**. All strains were normalized to the same OD and plated to each respective plate. After 48 h of growth at 35°C, CFU were counted and the Survival % calculated by comparing dividing counts by the YNB control plate CFU.

Table 1 Strains Tested for Antifungal Sensitivity

Strain ID	Description	Producer	Notes
[redacted]	Industrial distillers *Saccharomyces cerevisiae* strain FALI	ABMauri	Control
[redacted]	Industrial distillers *Saccharomyces cerevisiae* strain [redacted]	[redacted]	Control
[redacted]	*Saccharomyces cerevisiae* strain – unmodified strain	[redacted]	Test Strain
[redacted]	Bioengineered *Saccharomyces cerevisiae* strain	[redacted]	Test Strain
[redacted]	Bioengineered Saccharomyces cerevisiae strain	[redacted]	Test Strain
[redacted]	Bioengineered Saccharomyces cerevisiae strain	[redacted]	Test Strain

[1] Barchiesi, F., Arzeni, D., Compagnucci, P., di Francesco, L. F., Giacometti, A., & Scalise, G. (1998). *In vitro* activity of five antifungal agents against clinical isolates of *Saccharomyces cerevisiae*. *Medical Mycology*, *36*(6), 437–440.

Table 2 Minimal Inhibitor Concentration (mg/L) of Yeast Strains Described in Table 1		
Strain ID	**Amphotericin B**	**Fluconazole**
[redacted] control strain	0.5	14
[redacted] control strain	1	14
[redacted] unmodified strain	[redacted]	[redacted]
[redacted] bioengineered strain	[redacted]	[redacted]
[redacted] bioengineered strain	[redacted]	[redacted]
[redacted] bioengineered strain	[redacted]	[redacted]

Table 3. Raw Data for Fluconazole Platings				
Media	Replicate 1		Replicate 2	
	CFU	Survival %	CFU	Survival %
[redacted] control strain				
YNB	227	100	131	100
YNB + Fluconazole 8mg/L	228	100.4	112	85.5
YNB + Fluconazole 10mg/L	198	87.2	102	77.9
YNB + Fluconazole 12mg/L	66	29.1	39	29.8
YNB + Fluconazole 14mg/L	17	7.5	9	6.9
[redacted] control strain				
YNB	221	100	136	100
YNB + Fluconazole 8mg/L	212	95.9	94	69.1
YNB + Fluconazole 10mg/L	210	95.0	86	63.2
YNB + Fluconazole 12mg/L	183	82.8	84	61.8
YNB + Fluconazole 14mg/L	17	7.7	4	2.9
[redacted] unmodified strain				
YNB	[redacted]	[redacted]	[redacted]	[redacted]
YNB + Fluconazole 8mg/L	[redacted]	[redacted]	[redacted]	[redacted]
YNB + Fluconazole 10mg/L	[redacted]	[redacted]	[redacted]	[redacted]
YNB + Fluconazole 12mg/L	[redacted]	[redacted]	[redacted]	[redacted]
YNB + Fluconazole 14mg/L	[redacted]	[redacted]	[redacted]	[redacted]
[redacted] bioengineered strain				
YNB	[redacted]	[redacted]	[redacted]	[redacted]
YNB + Fluconazole 8mg/L	[redacted]	[redacted]	[redacted]	[redacted]
YNB + Fluconazole 10mg/L	[redacted]	[redacted]	[redacted]	[redacted]
YNB + Fluconazole 12mg/L	[redacted]	[redacted]	[redacted]	[redacted]
YNB + Fluconazole 14mg/L	[redacted]	[redacted]	[redacted]	[redacted]
[redacted] bioengineered strain				
YNB	[redacted]	[redacted]	[redacted]	[redacted]
YNB + Fluconazole 8mg/L	[redacted]	[redacted]	[redacted]	[redacted]
YNB + Fluconazole 10mg/L	[redacted]	[redacted]	[redacted]	[redacted]
YNB + Fluconazole 12mg/L	[redacted]	[redacted]	[redacted]	[redacted]
YNB + Fluconazole 14mg/L	[redacted]	[redacted]	[redacted]	[redacted]
[redacted] bioengineered strain				
YNB	[redacted]	[redacted]	[redacted]	[redacted]
YNB + Fluconazole 8mg/L	[redacted]	[redacted]	[redacted]	[redacted]
YNB + Fluconazole 10mg/L	[redacted]	[redacted]	[redacted]	[redacted]
YNB + Fluconazole 12mg/L	[redacted]	[redacted]	[redacted]	[redacted]
YNB + Fluconazole 14mg/L	[redacted]	[redacted]	[redacted]	[redacted]

CFU = colony forming units

Table 4. Raw Data for Amphotericin B Platings				
Media	Replicate 1		Replicate 2	
	CFU	Survival %	CFU	Survival %
[redacted] control strain				
YNB	131	100	95	100
YNB + Amphotericin 0.25mg/L	92	70.2	92	96.8
YNB + Amphotericin 0.5mg/L	0	0	8	8.4
YNB + Amphotericin 1mg/L	0	0	0	0
[redacted] control strain				
YNB	136	100	109	100
YNB + Amphotericin 0.25mg/L	109	80.1	102	93.6
YNB + Amphotericin 0.5mg/L	0	0	20	18.3
YNB + Amphotericin 1mg/L	0	0	0	0
[redacted] unmodified strain				
YNB	[redacted]			
YNB + Amphotericin 0.25mg/L	[redacted]			
YNB + Amphotericin 0.5mg/L	[redacted]			
YNB + Amphotericin 1mg/L	[redacted]			
[redacted]] bioengineered strain				
YNB	[redacted]			
YNB + Amphotericin 0.25mg/L	[redacted]			
YNB + Amphotericin 0.5mg/L	[redacted]			
YNB + Amphotericin 1mg/L	[redacted]			
[redacted] bioengineered strain				
YNB	[redacted]			[redacted]]
YNB + Amphotericin 0.25mg/L	[redacted]			[redacted]
YNB + Amphotericin 0.5mg/L	[redacted]			
YNB + Amphotericin 1mg/L	[redacted]			
[redacted] bioengineered strain				
YNB	[redacted]			[redacted]]
YNB + Amphotericin 0.25mg/L	[redacted]			
YNB + Amphotericin 0.5mg/L	[redacted]			
YNB + Amphotericin 1mg/L	[redacted]			

CFU = colony forming units

In conclusion, the wild-type [redacted]] and engineered strains [redacted]] are sensitive to clinical antifungal drugs. The minimal inhibitor concentrations (MIC) are at or below the controls when measured with two antifungal drugs typically used to treat yeast infections.