A Simple and Noninvasive Zebrafish Egg Infection Model for Studying Pathogenic Yeasts

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The prevalence of fungal infections increases significantly in the past two decades. Major infections (~70%) are caused by *Candida* species. *Candida albicans* is polymorphic yeast that grows as yeast form which can extend to hyphae or pseudohyphae form. Disseminated *candidiasis* is associated with 30–40% mortality in severely immunocompromised patients. We have established a simple, noninvasive zebrafish egg bath infection model, defined its optimal conditions, and evaluated the model with various *C. albicans* mutant strains. Wild-type zebrafish (*Danio rerio*), aged approximately 8 -15 months were maintained in the zebrafish core facility at National Health Research Institutes (NHRI). One day post-fertilization, the embryos were sterilized using 0.028% chlorine bleach containing 0.0017% sodium hypochlorite to reduce the possibility of contamination. In general, after co-incubation, non-adhered *Candida* cells were removed from embryos by washing with egg water 3 times. Embryos incubated in 1 mL of egg water in 24-well plates at 30 °C were imaged daily under an inverted microscope, with a beating heart used to indicate viability. The deletion of *SAP6* did not have significant effect on the virulence. By contrast, the deletion of *BCR1*, *CPH1*, *EFG1*, or *TEC1* significantly reduced the virulence under current conditions. Furthermore, all embryos survived when co-incubated with *bcr1/bcr1*, *cph1/cph1*, *efg1/efg1*, *efg1/efg1*, or *tec1/tec1* mutant cells. The results indicated that our novel zebrafish model is time-saving and cost effective. Recently, we extended our novel zebrafish egg infection model to study the pathogenesis of other common *Candida* species, including *C. glabrata* and *C. parapsilosis*.

Table 1. Optimal conditions for the zebrafish egg bath infection model

	1x10 ⁷ cells/ml			1x10 ⁸ cells/ml					
	RPMI 6h	EW ^{*1} 6h	RPMI 16 h	RPMI 4h	RPMI 6h	RPMI 16 h	EW ^{*2} 4h	EW 6h	EW 16 h
	Additional 2 days of incubation								
Control	100			100	100		100	100	
SC5314	0			0	0		10	33	
cph1 efg1	100			100	100		100	100	
C. krusei	100		80	80	100	100	100		100
C. parapsilosis	10		0	100	100	86	100		92
C. glabrata	98	82	100	80	83	100	0		27
C. tropicalis	94		56	82	100	100	100		100

^{*1} EW = Egg water 0.03% sea salt ^{*2} Percentage of embryos survival rate
^{*3} Number of embryos

Table 2. Embryos survival rates after co-incubated with pathogenic yeasts

	Additional incubation (day)	
	1	2
Control (a)	100	100
SC5314 (b)	0	0
C. krusei ATCC-Sputum (c)	100	86
C. krusei –oral (d)	100	100
C. krusei –oral (e)	100	100
C. parapsilosis ATCC (f)	20	0
C. parapsilosis –urine (g)	100	90
C. glabrata - EW ATCC-blood (h)	5	5
C. glabrata –oral – EW (i)	95	68
C. glabrata –urine- EW (j)	71	35
C. tropicalis ATCC (k)	94	94
C. tropicalis –oral (l)	10	0
C. tropicalis –oral (m)	0	0
C. tropicalis –urine (n)	89	89

This model can detect different level of pathogenesis within the same species.

Figure. Zebrafish egg infected by pathogenic yeasts from different sources. Representative embryos were co-incubated without yeast (a) or with 1 × 10⁷ cells/mL *C. albicans* (b), *C. krusei* (c-e), *C. parapsilosis* (f, g), and *C. tropicalis* (k-n), and in RPMI medium or in egg water (*C. glabrata*, h-j) at 30 °C for 6 h. Embryos were photographed after 2 additional days of incubation.

CONCLUSIONS

1. Recently, we have successfully established the zebrafish egg bath infection model in *C. albicans*. In the present study, we showed that this model can be extended to investigate the pathogenesis of other common pathogenic yeasts.
2. The optimal conditions are as follow: Yeasts 1x10⁷ cells/ml co-incubated with 1 day old embryos in RPMI (all tested species but *C. glabrata*) or egg water (*C. glabrata*) for 6 h at 30 °C . After washed, embryos were incubated in egg water containing 0.5% YPD for 2 days.
3. So far, we found that some *C. parapsilosis*, *C. glabrata*, and *C. tropicalis* isolates can adhere to chorion and cause embryo death.
4. We are investigating the mechanisms causing death of embryos.