Differential thermotolerance adaptation between species of *Coccidioides*

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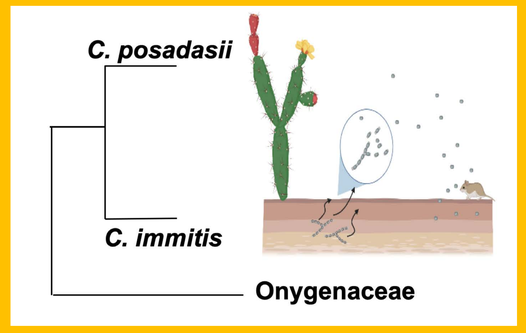
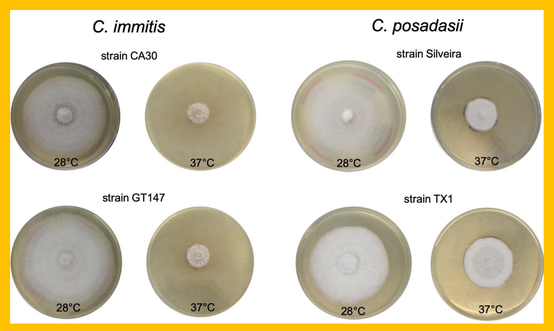
Introduction

Coccidioidomycosis, or Valley fever, is caused by two species of dimorphic fungi. Based on molecular phylogenetic evidence, the genus *Coccidioides* contains two reciprocally monophyletic species: *C. immitis* and *C. posadasii*. However, phenotypic variation between species has not been deeply investigated. We therefore explored differences in growth rate under various conditions. A collection of 39 *C. posadasii* and 46 *C. immitis* isolates, representing the full geographical range of the two species, were screened for mycelial growth rate at 37ºC and 28ºC on solid media.

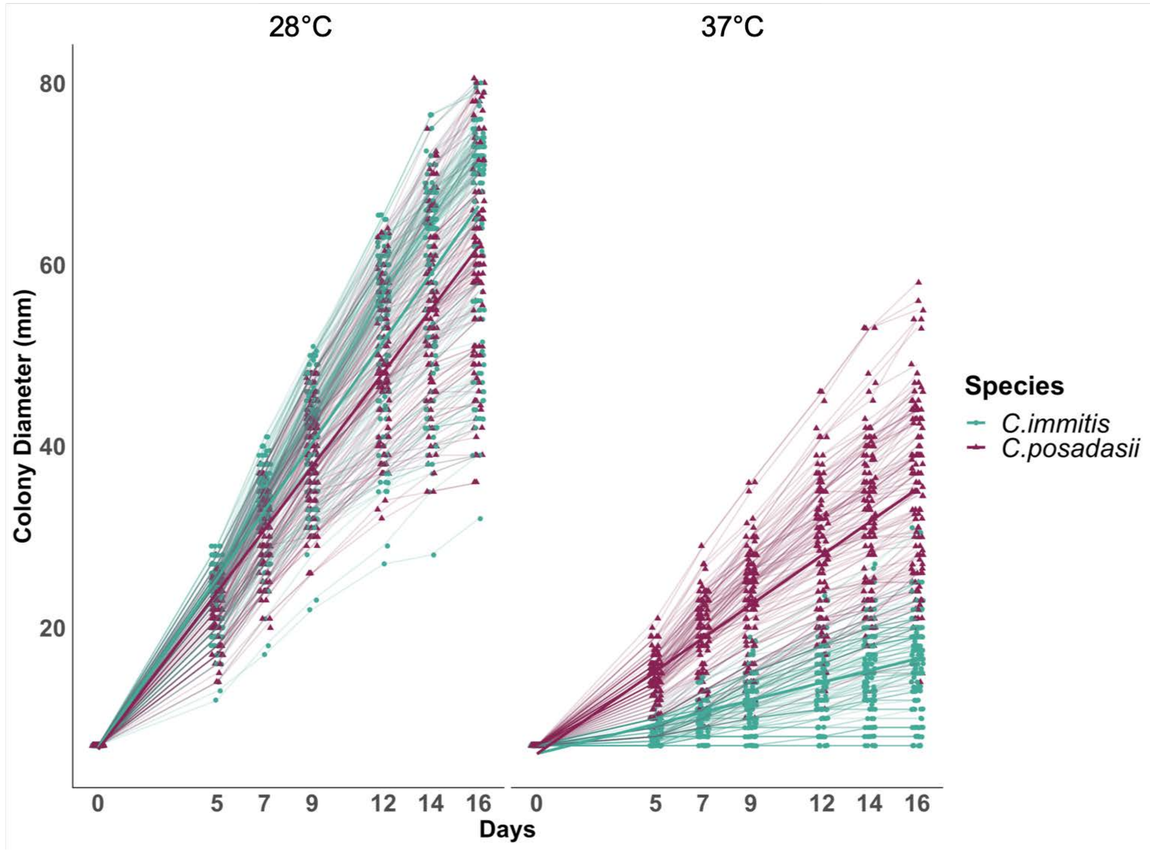


The genus *Coccidioides* are dimorphic fungi found in arid desert soil. These fungi are thought to have co-evoled with mammals, therefore they can tolerate environmental and host temperatures.



Methods

The radial growth rate was measured over 16 days (day 5,7,9,12,14,16) on yeast extract agar. A total of 86 isolates were grown in triplicate. A linear mixed effect model was used to compare the growth rate of *C. posadasii* and *C. immitis* at 37ºC and 28ºC respectively.



C. Posadasii grows at a faster rate than _C. immitis_ at 37C

Results

Table 1 summarizes the estimated growth rate for each species, 95% confidence interval (CI), and p-value for each temperature specific model. Both species grew quicker at 28°C than 37°C. Although, *C. posadasii* had a larger mean diameter on all days tested the overall rate of increase was not statistically significant (p-value = 0.072, Table 2).

This was in contrast to growth at 37°C. At this temperature, *C. posadasii* strains exhibited larger mean diameters, which reached double the diameter of *C. immitis* by day 16. At this temperature the overall growth rate of *C. posadasii* was 1mm/day faster than *C. immitis* (Figure 2 and Table 2). This difference was statistically significant (p-value < 0.001, Table 2). These findings were consistent for all days tested, and represent differential phenotypes for both species. Thus, our analysis indicates that high temperature is the important variable between species growth rate on solid media.

	Colony Diameter at 28ºC			Colony Diameter at 37ºC		
Species	*mm/day*	*95% CI*	*p*[a]	*mm/day*	*95% CI*	*p*[a]
C. immitis x Day	3.73	3.53 – 3.92	0.072	0.64	0.51 – 0.78	**<0.001**
C. posadasii x Day	3.47	0.55 – 0.02		1.82	0.98 – 1.38	
N[b]		85			85	

[a] difference between estimated slope [b] number of isolates

Table 1. Temperature Specific Linear Model Slope Estimates for Radial Growth Rate at 28ºC or 37ºC

Discussion

Our analysis indicates that high temperature is the important variable between species growth rate on solid media. This phenotypic difference supports the molecular phylogenetic species designation and may reflect adaptation of *C. immitis* to cooler environments, or possibly specific hosts. As the ecological niche has not been well-described for *Coccidioides* spp., and disease variability between species has not been shown, the evolutionary pressure underlying the adaptation is unclear. However, this research reveals the first significant phenotypic difference between the two species that directly applies to ecological and clinical research.