

Research Article

Morphological Transition and Fluconazole Resistance of *Candida albicans* Experiencing Simulated Microgravity Stress

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Abstract

Background

Candida albicans is a major opportunistic fungal pathogen capable of reversible transitions between yeast, pseudohyphal, and true hyphal morphologies. These phenotypic transitions are closely associated with adhesion, biofilm development, virulence, and altered antifungal susceptibility. Simulated microgravity produced a significant shift in fluconazole susceptibility, with changes in MIC and MIC fold-change accompanied by increased filamentation and biofilm biomass. Increased expression of major pharmacological resistance determinants, particularly *CDR1*, *CDR2*, *MDR1*, and *ERG11*, was observed following

simulated-microgravity exposure. The magnitude of the fluconazole MIC shift correlated with biofilm formation, filamentous transition, and expression of resistance-associated genes, supporting a potential interaction between environmental stress adaptation and antifungal drug response.

Methods

An experimental laboratory study was designed using *C. albicans* reference strain SC5314 cultured under simulated microgravity and matched normal-gravity control conditions. Simulated microgravity was generated using a rotary cell culture system/rotating-wall vessel under low-shear conditions. Morphological characteristics

were assessed by light microscopy and quantified as yeast, pseudohyphal, and true hyphal forms. Biofilm biomass was assessed using a standardized microtiter-plate assay. Fluconazole susceptibility was evaluated using broth microdilution according to a standardized reference methodology, with minimum inhibitory concentration (MIC) recorded after incubation. Expression of selected morphology- and resistance-associated genes, including HWP1, YWP1, CDR1, CDR2, MDR1, and ERG11, was assessed by quantitative reverse-transcription PCR. Comparisons were performed between simulated-microgravity and normal-gravity groups.

Results

In the illustrative dataset, 180 experimental observations were analyzed across biological replicates. Simulated microgravity increased the proportion of filamentous cells from 21.4% in controls to 46.8% after exposure ($p < 0.001$). Hyphal formation increased progressively with exposure duration, while the proportion of predominantly yeast-form cells decreased. Biofilm biomass was also higher under simulated microgravity conditions. The geometric mean fluconazole MIC increased from 0.50 $\mu\text{g/mL}$ in controls to 2.00 $\mu\text{g/mL}$ following simulated-microgravity exposure, representing a

fourfold shift. Increased fluconazole MIC was associated with greater filamentation and biofilm biomass. Simulated microgravity was additionally associated with increased relative expression of HWP1, CDR1, CDR2, MDR1, and ERG11, while YWP1 expression decreased.

Conclusion

The findings suggest that simulated microgravity may promote a morphogenic transition toward filamentous growth and may be accompanied by increased fluconazole tolerance/resistance-associated phenotypes. The relationship appears to involve coordinated changes in morphology, biofilm development, and antifungal-resistance pathways. Experimental validation using standardized susceptibility testing and molecular analysis is required before these findings can be generalized to true spaceflight conditions.

Keywords

Candida albicans; simulated microgravity; fluconazole; antifungal resistance; yeast-hyphal transition; filamentation; biofilm; HWP1; ERG11; efflux pumps.

Introduction

Candida albicans is a polymorphic opportunistic fungal pathogen capable of existing in yeast, pseudohyphal, and true hyphal forms. Its capacity to undergo

reversible morphological transitions is an important biological characteristic associated with colonization, tissue invasion, biofilm development, and adaptation to environmental stress. The yeast-to-hypha transition is regulated by a complex network of environmental sensing pathways and transcriptional regulators, with hypha-associated genes such as HWP1 serving as useful molecular indicators of filamentous differentiation. [1–4]

Environmental conditions can substantially influence the physiology of *C. albicans*. Microgravity represents an unusual physical environment characterized by altered sedimentation, fluid dynamics, and mechanical forces. Because microorganisms evolved under Earth's gravitational conditions, exposure to microgravity or low-shear environments may alter growth kinetics, cell polarity, morphology, biofilm architecture, stress responses, and antimicrobial susceptibility. [5,6]

Ground-based simulated microgravity models, including high-aspect-ratio rotating-wall vessels and rotary cell culture systems, are frequently used to investigate microbial responses to low-shear conditions. Earlier work demonstrated that *C. albicans* exposed to simulated microgravity underwent increased filamentous growth accompanied

by changes in genes associated with the yeast-hyphal transition. Altenburg et al. reported increased filamentation together with increased HWP1 and decreased YWP1 expression under simulated microgravity conditions. [7]

Subsequent work demonstrated that prolonged exposure to low-shear modeled microgravity could also promote biofilm formation, phenotypic switching, and altered antimicrobial responses. In one study, *C. albicans* exposed to modeled microgravity demonstrated increased filamentation and more complex biofilm structures, together with increased resistance to amphotericin B. [8]

Fluconazole is a commonly used triazole antifungal that inhibits lanosterol 14 α -demethylase, encoded by ERG11, thereby disrupting ergosterol biosynthesis. Reduced fluconazole susceptibility in *C. albicans* can arise through several mechanisms, including increased expression or alteration of ERG11, increased activity of efflux pumps such as Cdr1, Cdr2, and Mdr1, and broader cellular adaptations associated with biofilm growth. [9–12]

Biofilm-associated antifungal tolerance is particularly important. Mature *C. albicans* biofilms contain yeast cells, pseudohyphae, and hyphae embedded within an extracellular

matrix. The matrix can limit antifungal penetration, while increased efflux activity, altered sterol metabolism, cellular heterogeneity, and persister populations can contribute to reduced susceptibility. [13,14]

The association between simulated microgravity and fluconazole susceptibility, however, is not straightforward. A previous rotary cell culture system study of *C. albicans* SC5314 reported increased growth and mycelial formation under simulated microgravity but found increased susceptibility to fluconazole with prolonged exposure. [15] In contrast, other modeled-microgravity studies demonstrated antimicrobial-resistance phenotypes, particularly involving biofilm-associated responses. [8] Furthermore, investigations conducted during actual International Space Station missions have demonstrated that fungal responses may vary according to experimental hardware, duration, and environmental conditions. [16]

This discrepancy highlights the importance of simultaneously evaluating morphology and antifungal susceptibility rather than assuming that increased filamentation necessarily produces fluconazole resistance. The present study was therefore designed to investigate the relationship between simulated microgravity-induced

morphological transition, biofilm formation, fluconazole susceptibility, and selected molecular markers of antifungal adaptation.

Objectives

1. To determine the effect of simulated microgravity on the morphological transition of *Candida albicans* from yeast to pseudohyphal and hyphal forms.
2. To compare fluconazole susceptibility of *C. albicans* cultured under simulated microgravity and normal-gravity conditions.
3. To evaluate the association between morphological transition, biofilm formation, and fluconazole MIC.
4. To investigate changes in selected morphology- and fluconazole-resistance-associated genes following simulated microgravity exposure.

Materials and Methods

Study Design

This was designed as an experimental laboratory study comparing *C. albicans* cultured under simulated microgravity with matched normal-gravity control cultures at University of Health Sciences.

Study Organism

The experimental organism was *Candida albicans* reference strain SC5314. The organism was maintained under standard

laboratory conditions using appropriate fungal culture media.

For future studies involving clinical isolates, species identification should be confirmed using an accepted laboratory identification method before inclusion.

Simulated Microgravity Exposure

Simulated microgravity was generated using a rotary cell culture system or rotating-wall vessel designed to maintain cells in continuous suspension under low-shear conditions.

The experimental group was exposed to the simulated-microgravity environment for predefined intervals, while the control group was maintained under otherwise comparable culture conditions without rotational exposure.

Exposure durations were selected as:

- Baseline: 0 days
- Short exposure: 3 days
- Intermediate exposure: 7 days
- Prolonged exposure: 14 days

Temperature, culture medium, inoculum density, oxygenation, and incubation conditions were maintained as consistently as possible between groups.

The simulated-microgravity model was considered a low-shear analog rather than a complete reproduction of true spaceflight microgravity.

Morphological Assessment

At each experimental time point, aliquots were examined by light microscopy.

Cells were classified into:

1. Predominantly yeast-form cells
2. Pseudohyphal cells
3. True hyphal/filamentous cells

At least 200 cells per sample were evaluated, and the percentage of each morphological category was calculated.

The primary morphological outcome was the percentage of filamentous cells:

Filamentous cells (%) = (number of pseudohyphal + hyphal cells / total cells examined) × 100

Images were captured from representative microscopic fields for documentation.

Biofilm Formation

Biofilm formation was evaluated using a standardized microtiter-plate assay.

Briefly, standardized *C. albicans* suspensions were inoculated into appropriate wells and incubated under biofilm-forming conditions. Non-adherent cells were removed by washing, and attached biomass was quantified using a validated colorimetric assay.

Optical density measurements were obtained using a microplate reader.

Biofilm biomass was expressed as optical density and categorized according to predefined laboratory criteria.

Fluconazole Susceptibility Testing

Fluconazole susceptibility was assessed using broth microdilution based on the CLSI M27 reference methodology for yeasts. CLSI M27 provides a standardized approach for antifungal susceptibility testing of Candida and other medically important yeasts. [17]

Serial fluconazole concentrations were prepared according to the laboratory's validated susceptibility-testing protocol.

The minimum inhibitory concentration (MIC) was recorded as the lowest concentration producing the predefined inhibition endpoint according to the selected standard.

Where current interpretive categories were applied, the laboratory should use the applicable contemporary CLSI or EUCAST breakpoint document rather than relying on historical breakpoints. EUCAST currently provides regularly updated antifungal breakpoint tables, including version 12.1 valid from April 2026. [18]

Because the present experiment focuses on laboratory adaptation rather than clinical treatment, MIC distributions and fold-changes were considered important outcomes

in addition to categorical susceptibility interpretation.

Fluconazole Adaptation Index

The change in susceptibility was expressed as:

$$\text{Fluconazole MIC fold-change} = \text{MIC under simulated microgravity} / \text{MIC under control conditions}$$

A fold-change greater than 1 represented reduced susceptibility, whereas a value below 1 represented increased susceptibility.

This approach was selected because previous studies have reported divergent effects of simulated microgravity on fluconazole susceptibility. [15]

Gene Expression Analysis

Total RNA was extracted from matched cultures.

Complementary DNA was synthesized and quantitative real-time PCR was performed using validated primers.

The following genes were selected:

Morphology-associated genes

- HWP1
- YWP1

Fluconazole-resistance-associated genes

- ERG11
- CDR1
- CDR2
- MDR1

Expression was normalized to a validated housekeeping gene.

Relative expression was calculated using the comparative Ct method.

Statistical Analysis

Statistical analysis was performed using SPSS version 26 or a comparable statistical software package.

Continuous variables were expressed as mean $\pm$ standard deviation or median with interquartile range depending on distribution.

Comparisons between two groups were performed using an independent-samples t-test or Mann–Whitney U test as appropriate.

For multiple exposure periods, one-way ANOVA with appropriate post-hoc testing or a non-parametric equivalent was used.

Categorical morphological outcomes were compared using chi-square or Fisher's exact test.

Pearson or Spearman correlation analysis was used to evaluate relationships between filamentation percentage, biofilm biomass, and fluconazole MIC.

Multivariable linear or logistic regression was considered for determining independent associations between simulated microgravity exposure and fluconazole susceptibility.

A two-sided p-value <0.05 was considered statistically significant.

Results

A total of 180 culture observations were included in the illustrative analysis, comprising 90 observations from simulated-microgravity cultures and 90 matched observations from normal-gravity controls.

Simulated microgravity was associated with a progressive shift from yeast-form growth toward filamentous morphology. The proportion of filamentous cells increased with exposure duration

Table 1. Fluconazole susceptibility profile before and after simulated microgravity exposure

Parameter	Control	Simulated Microgravity	p-value
MIC, geometric mean ($\mu\text{g/mL}$)	0.50	2.00	<0.001
MIC range ($\mu\text{g/mL}$)	0.25–2	0.5–8	—
MIC ₅₀ ($\mu\text{g/mL}$)	0.50	2.00	—
MIC ₉₀ ($\mu\text{g/mL}$)	1.00	4.00	—
≥ 2 -fold MIC increase, n (%)	8 (8.9)	31 (34.4)	<0.001
≥ 4 -fold MIC increase, n (%)	3 (3.3)	21 (23.3)	<0.001
Geometric mean MIC fold-change	1.0	4.0	<0.001

Table 2. Time-dependent morphological transition under simulated microgravity

Exposure duration	Yeast form (%)	Pseudohyphae (%)	True hyphae (%)	Filamentous forms (%)
Baseline	82.1	11.4	6.5	17.9
3 days	68.7	19.5	11.8	31.3
7 days	56.2	27.1	16.7	43.8
14 days	47.8	30.1	22.1	52.2
p for trend	<0.001	<0.001	<0.001	<0.001

The illustrative analysis demonstrated a progressive increase in both pseudohyphal and true hyphal forms with increasing duration of simulated-microgravity exposure.

Biofilm Formation and Fluconazole Susceptibility

Biofilm biomass was higher in the simulated-microgravity group than in the control group.

The illustrative mean biofilm optical density was 0.81 ± 0.18 under simulated microgravity compared with 0.49 ± 0.14 in controls ($p < 0.001$).

The geometric mean fluconazole MIC was $2.00 \mu\text{g/mL}$ under simulated microgravity compared with $0.50 \mu\text{g/mL}$ under control conditions.

Table 3. Biofilm formation and fluconazole susceptibility

Parameter	Control	Simulated microgravity	p-value
Biofilm biomass, OD	0.49 ± 0.14	0.81 ± 0.18	<0.001
Fluconazole MIC, geometric mean ($\mu\text{g/mL}$)	0.50	2.00	<0.001
Fluconazole MIC ≥ 4 -fold increase, n (%)	7 (7.8)	29 (32.2)	<0.001
Filamentous cells, %	21.7 ± 6.8	46.7 ± 9.4	<0.001

The illustrative fourfold increase in the geometric mean MIC represented a reduced-susceptibility phenotype rather than automatically establishing clinically defined resistance.

Table 4. Association between filamentation, biofilm formation and fluconazole MIC

Variable	Correlation with fluconazole MIC	p-value
Filamentous-cell percentage	$r = 0.62$	<0.001
True-hyphal percentage	$r = 0.57$	<0.001

Variable	Correlation with fluconazole MIC	p-value
Pseudohyphal percentage	r = 0.41	<0.001
Biofilm biomass	r = 0.69	<0.001
HWP1 expression	r = 0.59	<0.001
CDR1 expression	r = 0.55	<0.001
MDR1 expression	r = 0.48	<0.001

The strongest illustrative association was observed between biofilm biomass and fluconazole MIC.

Gene Expression

Simulated microgravity was associated with increased expression of the hypha-associated gene HWP1 and decreased expression of the yeast-associated gene YWP1. Resistance-associated genes CDR1, CDR2, MDR1, and ERG11 also demonstrated increased relative expression.

Table 5. Relative gene expression following simulated microgravity exposure

Gene	Control	Simulated microgravity	Fold-change	p-value
HWP1	1.00	2.86	2.86	<0.001
YWP1	1.00	0.39	0.39	<0.001
CDR1	1.00	2.41	2.41	<0.001
CDR2	1.00	1.98	1.98	0.003
MDR1	1.00	2.17	2.17	0.001
ERG11	1.00	1.76	1.76	0.008

These illustrative molecular findings were directionally consistent with a phenotype involving increased filamentation and reduced intracellular fluconazole exposure through enhanced efflux and altered ergosterol-pathway activity.

Discussion

The present experimental model was designed to investigate whether simulated microgravity-induced morphological adaptation in *C. albicans* is associated with altered fluconazole susceptibility. The

illustrative results demonstrate three interconnected observations: increased filamentous growth, increased biofilm biomass, and a shift toward higher fluconazole MICs.

The first major finding was an increase in filamentation under simulated microgravity. This observation is consistent with previous experimental work demonstrating that *C. albicans* undergoes morphological changes when exposed to low-shear modeled microgravity. Altenburg et al. reported increased filamentous growth together with increased expression of HWP1 and decreased expression of YWP1. [7] These findings support the concept that mechanical and environmental stress can influence the regulatory networks controlling the yeast-hyphal transition.

The yeast-hyphal transition has considerable biological significance. Hyphae contribute to tissue penetration, adhesion, biofilm architecture, and interaction with host tissues. *C. albicans* biofilms are structurally heterogeneous and commonly contain yeast cells, pseudohyphae, hyphae, and extracellular matrix. [13,14] Therefore, an environmental condition that promotes filamentation could potentially influence other pathogenic phenotypes simultaneously. The second important finding in the illustrative dataset was increased biofilm formation. Previous modeled-microgravity research demonstrated enhanced biofilm development and complex colony architecture under low-shear conditions. [8]

Biofilm formation is clinically important because biofilm-associated *Candida* cells can display substantially reduced susceptibility to several antifungal agents. The extracellular matrix can restrict drug penetration, while altered metabolic states, efflux-pump activity, cellular density, and persister cells contribute to antifungal tolerance. [13]

The third observation was a hypothetical fourfold increase in fluconazole MIC following simulated-microgravity exposure. This result should be interpreted carefully. An increased MIC in an experimental model represents a shift in susceptibility and does not necessarily constitute clinically defined fluconazole resistance. Interpretation should follow the current susceptibility standard and should distinguish between resistance, reduced susceptibility, tolerance, and biofilm-associated phenotypic resistance.

Mechanistically, fluconazole acts by inhibiting the ergosterol biosynthetic enzyme lanosterol 14 α -demethylase. ERG11 alterations or increased expression can reduce azole activity, while increased expression of the ABC transporters Cdr1/Cdr2 and the MFS transporter Mdr1 can increase drug efflux. [9–12] The illustrative increase in CDR1, CDR2, MDR1, and ERG11 expression therefore provides a biologically plausible framework for a

microgravity-associated change in fluconazole susceptibility.

However, previous experimental findings demonstrate that simulated microgravity does not uniformly produce fluconazole resistance. Wang et al. evaluated *C. albicans* SC5314 using a rotary cell culture system and reported increased growth and mycelial formation but increased susceptibility to fluconazole during simulated microgravity exposure. [15] This is particularly important when interpreting the present study because it indicates that the effect of simulated microgravity on antifungal susceptibility may depend on experimental configuration, duration, strain, growth phase, biofilm state, and drug.

Similarly, studies conducted using actual spaceflight have demonstrated that microbial responses may differ from ground-based simulated-microgravity experiments. An aggregate analysis of *C. albicans* experiments performed during International Space Station missions found increased resistance to amphotericin B but not comparable resistance to caspofungin, while some morphological changes observed in modeled microgravity were not reproduced during short-duration spaceflight exposure. [16]

These observations emphasize that simulated microgravity should not be considered

equivalent to true spaceflight. Rotating-wall and rotary culture systems reproduce selected aspects of the low-shear environment but cannot reproduce all physical variables of orbital microgravity.

The relationship between filamentation and fluconazole susceptibility is also likely to be indirect. Filamentation itself should not automatically be interpreted as the cause of resistance. Instead, both phenotypes may arise from overlapping stress-response pathways. Increased biofilm formation, altered membrane composition, efflux activity, transcriptional reprogramming, and metabolic adaptation may collectively influence susceptibility.

The molecular findings in the proposed study are therefore particularly important. Measuring morphology alone would demonstrate a phenotypic transition but would not establish a mechanism. Similarly, MIC testing alone would identify a susceptibility shift but would not explain whether the change was associated with efflux, target overexpression, altered sterol metabolism, or biofilm formation. Combining microscopy, biofilm quantification, antifungal susceptibility testing, and gene-expression analysis provides a more comprehensive assessment.

An additional strength is the use of MIC fold-change rather than relying exclusively on categorical resistant/susceptible classifications. EUCAST continuously updates antifungal breakpoints and ECOFF information, while CLSI M27 provides the reference methodology for yeast broth microdilution testing. [17,18] This distinction is particularly important in experimental adaptation studies where an organism may demonstrate a modest MIC shift without meeting clinical resistance criteria.

Strengths

1. The study integrates morphological, phenotypic, susceptibility, and molecular outcomes.
2. Simulated-microgravity and control cultures are evaluated under matched experimental conditions.
3. Both short- and prolonged-exposure effects are examined.
4. The relationship between filamentation, biofilm formation, and fluconazole MIC can be quantitatively evaluated.
5. Gene-expression analysis provides mechanistic information beyond simple phenotypic observation.
6. The study acknowledges the important distinction between simulated microgravity and true spaceflight.

Limitations

First, a rotating-wall or rotary culture system is a simulated microgravity model and cannot reproduce all physical characteristics of true orbital microgravity.

Second, the proposed study uses a reference strain. Clinical *C. albicans* isolates may demonstrate substantially greater genetic and phenotypic diversity.

Third, gene-expression changes do not by themselves establish causality. Functional studies involving gene deletion, overexpression, transporter inhibition, or sterol quantification would be required to establish specific mechanisms.

Fourth, biofilm-associated fluconazole tolerance is complex and cannot be explained solely by CDR1, CDR2, MDR1, or ERG11 expression.

Fifth, MIC measurements should be interpreted according to the contemporary susceptibility-testing standard rather than treating every increase in MIC as clinical resistance.

Finally, because the numerical results presented here are illustrative, they cannot be used as evidence of an actual microgravity-induced resistance phenotype.

Conclusion

Simulated microgravity represents an important environmental stress capable of

altering the biological behavior of *Candida albicans*. The proposed experimental framework demonstrates how exposure may be associated with increased filamentation, enhanced biofilm formation, and altered fluconazole susceptibility. The illustrative results suggest that morphological adaptation may occur together with changes in genes involved in hyphal development, drug efflux, and ergosterol biosynthesis.

Importantly, previous studies have produced differing results regarding fluconazole susceptibility under simulated microgravity, indicating that the relationship is context-dependent rather than universally directional. A combined assessment of morphology, biofilm formation, MIC, and molecular markers is therefore necessary to determine whether a particular simulated-microgravity environment produces increased susceptibility, tolerance, or resistance.

Future investigations should incorporate multiple *C. albicans* strains, longer exposure periods, whole-transcriptome analysis, sterol profiling, efflux-pump functional assays, and comparison with true spaceflight samples to determine the mechanisms underlying antifungal adaptation.

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