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Paradoxical Growth Effect of Echinocandins in Fungal Biofilms: A Literature Review on Mechanisms, Species Specificity, and Clinical Relevance

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ABSTRACT

The paradoxical growth (PG) effect, also termed the Eagle-like effect, describes the resumption of fungal growth at echinocandin concentrations above the minimum inhibitory concentration (MIC), contrary to the expected dose-dependent inhibition. Although documented for nearly two decades, the relevance of PG to biofilm-associated infections and its clinical significance remain incompletely understood. This literature review examines the evidence for PG in fungal biofilms, encompassing both *Candida* and *Aspergillus* species, with emphasis on mechanisms, species-specific prevalence, and therapeutic implications. A narrative literature review was conducted by searching PubMed and Scopus for studies published between 2004 and 2025, with priority given to the last decade. The evidence indicates that PG occurs in sessile cells of multiple *Candida* species, with prevalence varying by species and echinocandin type; *C. parapsilosis* and *C. albicans* exhibit the highest rates with caspofungin, whereas *C. glabrata* appears uniformly non-paradoxical. In *Aspergillus fumigatus*, PG is observed with caspofungin but not consistently with micafungin or anidulafungin. Mechanistically, PG involves compensatory cell wall remodeling, particularly chitin deposition, and activation of stress-response pathways including calcineurin and Hsp90. Recent evidence implicates segmental aneuploidy as a reversible adaptive mechanism in *C. albicans*. Farnesol eliminates PG *in vitro*, suggesting a potential adjunctive strategy. The clinical relevance of PG remains uncertain due to limited *in vivo* confirmation and the high drug concentrations required *in vitro*. Standardized detection methods and clinical correlation studies are needed before treatment recommendations can be modified.

Keywords: paradoxical growth; Eagle effect; echinocandin; fungal biofilm; *Candida*; *Aspergillus*

INTRODUCTION

Echinocandins are first-line agents for invasive candidiasis and are recommended by the Infectious Diseases Society of America for the treatment of candidemia and other forms of invasive candidiasis [1]. They are recognized for their activity against *Candida* biofilms, which are notoriously resistant to azoles and polyenes [2]. Their mechanism involves non-competitive inhibition of β-1,3-D-glucan synthase, leading to cell wall depletion and osmotic instability [3]. However, since the early 2000s, an anomalous phenomenon has been documented: at concentrations above the MIC, some fungal isolates resume growth, a behavior termed paradoxical growth (PG) or the Eagle-like effect [4,5].

PG was initially described in planktonic cells, but subsequent studies demonstrated that sessile cells within biofilms also exhibit this behavior [6,7]. The phenomenon has been observed across multiple *Candida* species and in *Aspergillus fumigatus*, though with substantial species- and drug-specific variability [8,9]. Despite two decades of investigation, fundamental questions remain: What is the clinical relevance of PG? Does it occur at achievable drug concentrations *in vivo*? And what are the implications for biofilm-associated infections, particularly in the context of antifungal lock therapy where high local echinocandin concentrations are intentionally used [10]?

Recent studies have begun to unravel the molecular and genomic mechanisms underlying PG, including the role of chitin synthesis, stress-response pathways, and even reversible chromosomal alterations [11–15]. In parallel, the clinical significance of PG continues to be debated, with some authors arguing that its impact may be overestimated [5,16]. This review synthesizes the current evidence on PG in fungal biofilms, with emphasis on recent findings regarding mechanisms, species-specific prevalence, and potential clinical implications.

MATERIALS AND METHODS

Search Strategy and Information Sources

This literature review was conducted by searching for primary and secondary literature across electronic databases, including PubMed and Scopus. The search included studies published between 2004 and 2025, with priority given to studies from the last decade (2016–2025) to capture recent advances. Foundational studies from earlier years were included when they provided essential mechanistic context not superseded by more recent work. The search strategy utilized combinations of the following terms: "paradoxical growth," "Eagle effect," "echinocandin," "biofilm," "Candida," "Aspergillus," and "cell wall stress."

Eligibility Criteria

Studies were selected based on predefined inclusion and exclusion criteria:

Inclusion Criteria:

1) Peer-reviewed original research articles, systematic reviews, and methodological protocols.
2) Studies evaluating PG in *Candida* or *Aspergillus* species, with specific relevance to biofilm or sessile cells.
3) Studies investigating mechanisms, species-specific prevalence, or therapeutic strategies targeting PG.

Exclusion Criteria:

1) Studies focusing exclusively on planktonic cells without biofilm relevance.
2) Conference abstracts without full text or non-peer-reviewed commentaries.

Data Synthesis

Given the heterogeneity of the included studies regarding experimental models, echinocandin types, and species, a thematic narrative synthesis was performed. Extracted findings were categorized into three domains: (1) evidence of PG in biofilms across species; (2) mechanisms underlying PG; and (3) clinical implications and therapeutic strategies.

EVIDENCE OF PARADOXICAL GROWTH IN FUNGAL BIOFILMS

Candida species

The earliest systematic evaluation of PG in biofilms was conducted by Melo et al., who examined caspofungin against biofilms formed by five *Candida* species. They demonstrated that PG occurred in sessile cells and that its frequency varied by species [6]. A subsequent study by Prażyńska et al. assessed PG in sessile cells of 70 clinical *Candida* isolates (*C. albicans*, *C. parapsilosis*, and *C. glabrata*) exposed to caspofungin. PG was observed in 26.9% to 93.1% of isolates, depending on species and sessile cell age [7].

Species-specific patterns have been documented. In a study of 60 bloodstream isolates, the prevalence of PG with caspofungin was 90% in *C. parapsilosis*, 60% in *C. albicans*, 40% in *C. tropicalis*, and 10% in *C. krusei*. Notably, PG was absent in all tested *C. glabrata* isolates for all three echinocandins (caspofungin, micafungin, and anidulafungin), a statistically significant finding [8]. For micafungin, PG was observed only in *C. tropicalis* (70%) and *C. krusei* (60%); for anidulafungin, only in *C. albicans* (40%) and *C. tropicalis* (20%) [8]. Similar echinocandin-specific patterns were reported by Fleischhacker et al., who found PG with caspofungin and micafungin but not anidulafungin in *C. albicans* and *C. dubliniensis* [19].

More recent evidence extends these findings to *Candida auris*. However, the cell wall dynamics in *C. auris* appear distinct from those in *C. albicans*, with only slight chitin accumulation observed during PG [11]. This species-specific difference suggests that the mechanisms underlying PG may not be uniform across *Candida* species.

Aspergillus species

PG in *Aspergillus fumigatus* is primarily associated with caspofungin and is not consistently observed with micafungin or anidulafungin [12]. The phenomenon has been demonstrated both *in vitro* and in a murine model of pulmonary aspergillosis, although it was not reproduced in a systemic candidiasis mouse model using strains that displayed PG *in vitro* [13]. Loiko and Wagener demonstrated that the paradoxical effect in *A. fumigatus* relies on recovery of the β-1,3-glucan synthase Fks1, providing a mechanistic explanation for the drug-specific nature of PG in this species [20].

MECHANISMS UNDERLYING PARADOXICAL GROWTH

The best-characterized mechanism of PG involves compensatory cell wall remodeling. In a *Candida* strain exhibiting PG, β-1,3-glucan content declined by 81% in the presence of caspofungin, while chitin content increased by 898%, compensating for the loss of glucan and allowing cell survival and growth [14]. This established chitin synthesis as a rescue mechanism against echinocandin-induced cell wall stress.

Subsequent studies confirmed and extended this observation. In PG cells of four *Candida* species, β-1,3-glucan decreased 2.7- to 7.8-fold while chitin increased 4.0- to 6.6-fold. Electron microscopy revealed enlarged cells, abnormal septa, and chitin accumulation in the cell wall [9]. Rueda et al. further demonstrated that PG cells of *C. albicans* exhibited decreased virulence in a *Galleria mellonella* infection model and induced proinflammatory responses in

murine macrophages, suggesting that the adaptive changes required for PG may impose a fitness cost [17].

Beyond cell wall remodeling, stress-response pathways play essential roles. In *C. albicans*, pharmacological inhibition or genetic deletion of Hsp90, PKC, calcineurin, or TOR abolished PG, confirming their involvement in adaptive stress responses [15]. In *A. fumigatus*, caspofungin at high concentrations induces a transient increase in cytosolic calcium, activating the calmodulin-calcineurin signaling pathway, which is required for PG [12]. The role of these pathways in echinocandin tolerance is further supported by studies on antifungal resistance mechanisms [23,24].

A recent study identified a novel genetic mechanism: segmental aneuploidy. Whole-genome sequencing of caspofungin-tolerant *C. albicans* isolates revealed a recurrent segmental monosomy on the right arm of Chromosome R. This aneuploidy was associated with upregulated expression of *GSC1* (β-1,3-glucan synthase) and chitin synthases, and phenotypic reversion analyses demonstrated that caspofungin resistance is reversible and directly linked to the presence of this chromosomal alteration [15]. This finding suggests that PG involves not only transient transcriptional responses but also reversible genomic plasticity.

CLINICAL IMPLICATIONS AND THERAPEUTIC STRATEGIES

The clinical relevance of PG remains uncertain. Vanstraelen et al. noted that although PG has been observed in numerous *in vitro* studies, confirming *in vivo* data are scarce, and "more and more data suggest that the clinical impact of this phenomenon might be heavily overrated" [5]. The high drug concentrations required to induce PG *in vitro* often exceed those achieved systemically, although local concentrations during antifungal lock therapy may fall within this range [10]. The clinical significance may also be species-dependent, given the variability in PG prevalence and the association of *C. glabrata* with echinocandin resistance through FKS mutations rather than PG [24,25].

One potential strategy to mitigate PG is combination therapy. Farnesol, a quorum-sensing molecule, eliminated PG induced by both caspofungin and micafungin in *C. parapsilosis* biofilms [16]. This finding suggests that targeting the cell wall stress response pathway may enhance echinocandin efficacy, though *in vivo* validation is needed. Similarly, inhibitors of the Hsp90-calcineurin axis have shown promise in potentiating echinocandin activity [12]. The potential for combination strategies is supported by broader reviews of antifungal resistance and tolerance mechanisms [22,26].

The clinical significance of PG may be more relevant for biofilm-associated infections than for planktonic infections, given the high cell densities and altered metabolic states within biofilms. However, direct evidence linking *in vitro* PG to treatment failure in biofilm-associated candidiasis or aspergillosis is lacking. The epidemiology of echinocandin resistance, including both FKS-mediated resistance and tolerance phenomena such as PG, continues to evolve [27–30].

DISCUSSION

This review synthesized evidence on PG in fungal biofilms, encompassing both *Candida* and *Aspergillus* species. The findings indicate that PG is a well-documented *in vitro* phenomenon with species- and drug-specific patterns. Mechanistically, it involves compensatory chitin synthesis, activation of stress-response pathways, and, in some isolates, reversible genomic alterations such as segmental aneuploidy. The role of cell wall integrity pathways in PG is consistent with broader mechanisms of echinocandin tolerance and resistance [23,24].

Several limitations of this review should be acknowledged. First, the heterogeneity of included studies varying in species, echinocandin type, biofilm model, and PG definition precluded a meta-analysis. Second, many mechanistic studies used planktonic cells or a limited number of laboratory strains, and extrapolation to clinical biofilm infections requires caution. Third, the search was restricted to studies published between 2004 and 2025, with priority given to the last decade; foundational studies from earlier years were cited where necessary for mechanistic context.

The clinical implications of PG remain unresolved. The discrepancy between *in vitro* observations and the scarcity of *in vivo* confirmation suggests that PG may be a laboratory phenomenon with limited clinical relevance. However, the use of high-dose echinocandin lock therapy for catheter-associated infections warrants continued attention, as local drug concentrations may reach PG-inducing levels [10]. The emergence of echinocandin-resistant *C. auris* and other non-albicans species further complicates the clinical picture [28–30].

Future research should prioritize: (1) standardized methods for detecting PG in biofilm models; (2) clinical studies correlating *in vitro* PG with treatment outcomes; and (3) evaluation of combination strategies (e.g., farnesol, Hsp90 inhibitors) in biofilm-associated infections.

CONCLUSION

Paradoxical growth of echinocandins in fungal biofilms is a well-documented *in vitro* phenomenon with a clear mechanistic basis in cell wall remodeling, stress-pathway activation, and, in some isolates, reversible genomic alterations. Species-specific prevalence varies widely, with *C. parapsilosis* and *C. albicans* exhibiting the highest rates with caspofungin and *C. glabrata* appearing uniformly non-paradoxical. In *Aspergillus fumigatus*, PG is primarily associated with caspofungin. The clinical *relevance of PG remains uncertain, and confirmatory in vivo* data are needed before treatment recommendations can be modified.

Farnesol and cell wall stress-modulating agents represent promising adjunctive strategies. Standardized detection methods and clinical correlation studies are necessary to resolve this long-standing question.

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