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Genetic Regulation and Development of *Candida albicans* Biofilms: A Literature Review

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A B S T R A C T

Candida albicans is a polymorphic fungal pathogen capable of forming biofilms on medical devices and host tissues, leading to persistent infections with high morbidity and mortality. The genetic regulation of biofilm formation involves complex transcriptional networks that orchestrate the temporal expression of genes throughout distinct developmental stages: initial adhesion, proliferation and filamentation, maturation, and dispersal. This literature review examines the genetic mechanisms controlling Candida albicans biofilm development, focusing on key transcriptional regulators including Bcr1p, Efg1p, Tec1p, and Rlm1p that coordinate stage-specific gene expression. These master regulators control the expression of adhesin gene families (Als1-Als8, Hwp1), morphogenesis-related genes, and extracellular matrix components essential for biofilm architecture and stability. Quorum sensing molecules such as farnesol and tyrosol function as genetic switches that modulate biofilm-related gene expression in response to cell density and environmental conditions. The genetic architecture underlying biofilm formation demonstrates remarkable complexity, with distinct expression profiles characterizing each developmental phase and contributing to enhanced antifungal resistance and immune evasion. Understanding these genetic regulatory mechanisms provides valuable insights into biofilm biology and identifies potential therapeutic targets for developing novel antifungal strategies that disrupt biofilm formation at the molecular level. This review synthesizes current knowledge of genetic regulation in Candida albicans biofilms and emphasizes the importance of targeting essential regulatory pathways to improve treatment outcomes for biofilm-associated candidiasis.

Keywords: Biofilm Pathogenesis, Antimicrobial Resistance, Host Immune Evasion, Persister Cells, Infectious Disease.

Introduction

The discovery of microorganisms under the microscope marked a pivotal moment in microbiology, laying the foundation for understanding microbial communities and their role in human health. One of the earliest observations of microbial aggregates was made by Antonie van Leeuwenhoek, who reported the presence of organisms on tooth surfaces—what we now recognize as biofilms (Flemming & Wingender, 2010).

Biofilms are structured communities of microorganisms that adhere to surfaces and are embedded in a self-produced extracellular polymeric substance (EPS) matrix. This matrix serves not only as a protective barrier but also as a scaffold that allows for structural organization, nutrient retention, and resistance to environmental threats (Flemming & Wingender, 2010). The transition of microorganisms from a free-living (planktonic) state to a sessile, surface-attached community is associated with significant phenotypic changes, including altered gene expression, reduced growth rate, and increased resistance to antibiotics (Donlan & Costerton, 2002).

Biofilms are now recognized as critical players in the pathogenesis of various infectious diseases. According to current estimates, biofilms are involved in more than 65% of microbial infections and up to 80% of chronic infections, especially those associated with indwelling medical devices such as urinary catheters, vascular grafts, and prosthetic joints (Hall-Stoodley et al., 2004; Jamal et al., 2018). Microorganisms in biofilms exhibit enhanced resistance to both antimicrobial agents and host immune responses, often making infections persistent and difficult to eradicate through conventional therapies (Costerton et al., 1999; Lewis, 2001).

This literature review aims to provide an integrative synthesis of current knowledge on biofilm structure, formation, and genetic regulation, particularly in Candida albicans, and to explore the clinical implications of biofilm-associated infections. Additionally, the review will highlight the role of quorum sensing and the emergence of antimicrobial resistance, with emphasis on the urgent need for novel therapeutic strategies to manage biofilm-related diseases.

Biofilm Structure and Composition

Biofilms consist of microbial cells and extracellular polymeric substances (EPS). The EPS, comprising 50% to 90% of the total organic carbon in biofilms, serves as the primary matrix material and plays a crucial role in biofilm integrity. Although EPS can exhibit varied chemical and physical properties, it predominantly consists of polysaccharides. In Gram-negative bacteria, these polysaccharides are typically neutral or polyanionic due to the presence of uronic acids such as D-glucuronate, D-galacturonic, and mannuronic acid. The anionic nature facilitates the association of divalent cations like calcium and magnesium, which cross-link with polymer strands to enhance binding strength in biofilm formation (Flemming & Wingender, 2010; Sutherland, 2001).

The chemical composition of EPS in Gram-positive bacteria, such as *Staphylococci*, differs significantly and tends to be cationic. EPS exhibits varying degrees of hydrophobicity, with some being predominantly hydrophilic whilst others demonstrate both hydrophilic and hydrophobic properties. This variation in hydrophobicity affects the solubility of the EPS and consequently influences biofilm formation and stability. Sutherland notes two important properties of EPS that may have marked effects on biofilms. First, the composition and structure of polysaccharides determine the primary confirmation of EPS; many bacterial EPS have structures with 1,3- or 1,4-β-hexose residues and tend to be more rigid, less deformable, and in certain cases insoluble or poorly soluble, while other EPS molecules may be readily soluble in water. Second, EPS from biofilms are generally not uniform but may vary spatially and temporarily (Sutherland, 2001).

Despite sharing common structural features, each biofilm community possesses unique characteristics. The term 'biofilm' may be somewhat misleading as biofilms do not form continuous monolayers on surfaces. Instead, they exhibit significant heterogeneity, comprising microcolonies of bacterial cells encased in EPS matrix and separated from one another by interstitial voids or water channels. These water channels serve as primitive circulatory systems, facilitating the exchange of nutrients and waste products within the biofilm community. The architectural heterogeneity of biofilms contributes to their functionality, allowing for the development of microenvironments with varying conditions of pH, oxygen concentration, and nutrient availability. This spatial organisation enables different bacterial species to occupy specific niches within the biofilm, promoting interspecies interactions and metabolic cooperation (Donlan, 2002; Tolker-Nielsen & Molin, 2000).

The formation of bacterial and fungal biofilms serves to protect microorganisms from adverse environmental conditions whilst ensuring access to essential nutrients. The process involves several complex stages, including cell attachment, cell-to-cell adhesion, proliferation and cell development, maturation, and dispersal (Sharma et al., 2023). The initial stage in biofilm formation involves the attachment or adhesion of cells to surfaces. Using *Candida albicans* as a model organism, biofilm development begins with the adherence of round yeast cells to solid surfaces such as silicone discs, intravascular catheters, or microtitre plates. This process typically occurs within 60-90 minutes, after which loosely attached cells are removed, resulting in a basal layer of adherent yeast cells. This 'seeding' step is critical for normal biofilm development (McCall et al., 2019).

During the initial attachment stage, bacterial cells interact with other microorganisms through weak van der Waals forces, allowing for reversible interactions where bacterial cells can separate and reconnect. Subsequently, specific adhesion involving hydrophilic or hydrophobic interactions occurs between attachment organelles and the surface, rendering the bacterial attachment irreversible (Mayton et al., 2021). Various factors, including nutrition, motility structures, temperature, carbohydrates, and proteins, can influence the rate of attachment (Sharma et al., 2023). The substratum (surface) characteristics significantly impact attachment efficiency. Attachment occurs more effectively on rough surfaces, which reduce the flow forces that could detach biofilm and provide a larger surface area. Microorganisms also adhere better to hydrophobic surfaces like Teflon and plastic compared to glass or metal (Costerton et al., 1999).

Surfaces exposed to liquid media will quickly be covered by polymers from the medium, creating a conditioning film that modifies the chemical properties and affects the growth and expansion of microorganism attachment to that surface. For example, tooth enamel becomes coated with a proteinaceous film called 'acquired pellicle' to which bacterial cells attach within hours of exposure. Following initial attachment, cell-to-cell adhesion occurs, where individual bacterial cells begin to adhere to one another, forming dense microcolonies. The next stage involves bacterial proliferation and development, where cells within microcolonies multiply in response to chemical signals that stimulate exopolysaccharide production once a certain threshold is reached. Bacterial cells continue to divide within the formed exopolysaccharide matrix (Sharma et al., 2023).

The subsequent stage is cell maturation, characterised by the expression of specific genes resulting in biofilm formation alongside the development of water channels. These channels function to transport nutrients into the biofilm and remove potentially harmful substances (Sharma et al., 2023). A mature biofilm typically forms within 24 hours and can be seen by the naked eye as an opaque surface structure over a solid surface, and under the microscope as a collection of various organized cell types. As the biofilm matures, it may contain not only bacteria but also fungi, algae, protozoa, tissue debris, and corrosion products from pipes. When bacteria live side by side, sometimes one species requires the metabolites of another species, creating mutual dependencies (Gulati et al., 2018).

The final stage of biofilm formation is cell dispersal. During this phase, bacteria cease EPS production, leading to the release of bacteria from the biofilm. These bacteria then disperse and develop in new environments, whilst the formed biofilm maintains properties that confer resistance to antibiotics ((Sharma et al., 2023). This dispersal mechanism allows bacterial populations to colonize new surfaces and establish additional biofilm communities, contributing to the persistence and spread of biofilm-associated infections in clinical settings and industrial environments.

Genetic Regulation of *Candida albicans* Biofilm Formation

The genetic regulation of *Candida albicans* biofilm formation involves a network of six "master" transcription regulators (Efg1, Tec1, Bcr1, Ndt80, Brg1, and Rob1), each essential for normal biofilm development both in vitro under standard laboratory conditions and in vivo in rat catheter and denture models. These regulators target various genes involved in different biofilm development processes, including adhesion, hyphal formation, drug resistance, and extracellular matrix production, which are critical characteristics of biofilms. Beyond these six master transcription regulators, an additional 44 transcription regulators have been identified as influencing at least some aspects of *Candida albicans* biofilm formation (Mancera et al., 2021).

The ability of *Candida albicans* to form biofilms on both biotic and abiotic surfaces significantly contributes to the resilience of *Candida albicans* biofilms, as shown in Figure 1. The initial attachment process begins when cells adhere to each other and to hard surfaces such as biomaterials in prosthetic devices or denture surfaces, or to soft surfaces like the epithelial mucosal lining in the oral cavity or vagina. This step represents the first phase in *Candida albicans* biofilm formation and is crucial for all subsequent stages of biofilm development. The master regulator Bcr1, along with its downstream targets including the cell wall proteins Als1, Als3, and Hwp1, are all necessary for attachment during biofilm formation. Additionally, several other transcription regulators are involved in attachment, with 30 transcription regulators identified as essential for adhesion to silicone substrates. Of these 30 regulators, four (Bcr1, Ace2, Snf5, and Arg81) are also required for biofilm formation under commonly used in vitro conditions (Gulati & Nobile, 2016).

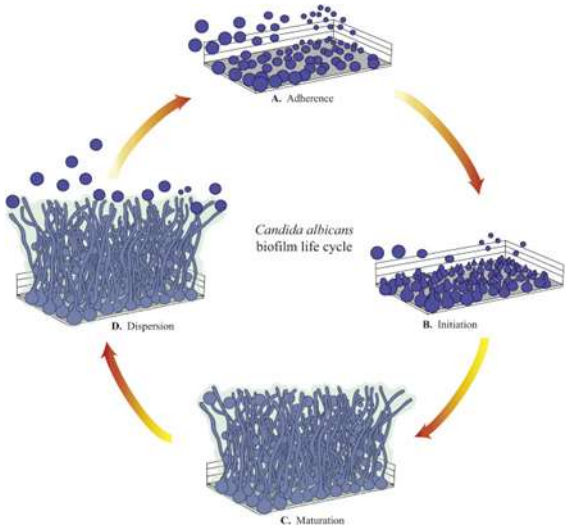

Figure 1. Life cycle of *Candida albicans* biofilm. A. Attachment of round yeast cells to a surface. B. Initiation of biofilm formation, where cells proliferate to form a basal layer of adherent cells. C. Biofilm maturation, during which a complex layer of polymorphic cells develops and becomes encased in an extracellular matrix. D. Dispersal, where round yeast cells leave the mature biofilm to seed new sites (Gulati & Nobile, 2016).

Following the initial adhesion of round yeast cells to surfaces to form a basal layer, the next phase of biofilm development involves the growth and proliferation of hyphal cells. *Candida albicans* is distinguished from many other fungal species by its ability to form both yeast and hyphal cells under various environmental conditions. *Candida albicans* is a polymorphic fungus, with *hyphae* being characteristic and important structural components of *Candida albicans* biofilms. Consequently, proteins involved in hyphal growth in suspension culture are also required for proper biofilm formation, including the transcription regulators Efg1, Tec1, Ndt80, and Rob1. *Hyphae* in biofilms contribute to the overall stability of the biofilm architecture and act as scaffolds for yeast cells, *pseudohyphae*, other *hyphae*, and other microbial cells in polymicrobial biofilm contexts. Thus, both the ability to form *hyphae* and the ability of these hyphae to adhere to each other and to other cell morphologies are essential for the development and maintenance of normal biofilms. The master regulator Bcr1 is required for *hyphae* to adhere to each other within the context of a biofilm (Gulati & Nobile, 2016; Malinovská et al., 2023).

A key feature of *Candida albicans* biofilms is the presence of an extracellular matrix that forms during the maturation phase of biofilm development. This matrix envelops the complex network of yeast cells, *pseudohyphae*, and *hyphae*, providing protection from host immune defences and antifungal drugs, whilst contributing to the stability of the three-dimensional biofilm architecture. Although the extracellular matrix is partly self-produced and secreted by *Candida albicans* cells within the biofilm, it can also contain environmental aggregates, such as structural components from lysed *Candida albicans* and host cells, including erythrocytes, epithelial cells, urothelial cells, and neutrophils, thus varying significantly depending on the location of the biofilm within the host (Nett et al., 2015). In vivo research has identified fourteen abundant host proteins in the matrix, including heme-related proteins and leukocyte-associated inflammatory proteins such as haemoglobin, myeloperoxidase, C-reactive protein, and S100-A9 alarmin. Several studies have examined the composition of *Candida albicans* biofilm matrix in vitro, finding that it predominantly consists of glycoproteins (55%), carbohydrates (25%), lipids (15%), and nucleic acids (5%) (Zarnowski et al., 2014).

Polysaccharides form the second largest fraction of the matrix, comprising glucose, mannose, rhamnose, and N-acetylglucosamine, with the largest fraction primarily consisting of mannan-glucan complexes mainly composed of α-1,6-linked mannan with α-1,2-linked side chains complexed with β-1,6-glucan. Over 500 proteins have been identified in the matrix, most of which are predicted to be enzymes, including hydrolytic enzymes, suggesting

that the matrix may play an active role in breaking down biopolymers. The biofilm matrix likely functions as an enzymatically active extracellular element of *Candida albicans* biofilms, capable of breaking down molecules both as a protective response and as a nutrient source (Zarnowski et al., 2014).

In vitro observations of biofilm development indicate that cells may disperse continuously during biofilm formation, typically in the form of round yeast cells. Although these dispersed cells morphologically resemble the round yeast cells seen in planktonic growth mode, they possess distinct characteristics. For instance, dispersed cells exhibit enhanced adhesion properties and have a higher capacity to form biofilms compared to planktonic cells (Uppuluri et al., 2010). Several transcription regulators of *Candida albicans* biofilm dispersal have been identified, including Nrg1, Pes1, and Ume6. Overexpression of UME6 reduces the number of dispersed cells, whereas overexpression of PES1 and NRG1 increases the number of dispersed cells actively released from biofilms (Uppuluri et al., 2010). The molecular chaperone Hsp90 has also been implicated in *Candida albicans* biofilm dispersal, as depletion of Hsp90 significantly reduces the number of cells dispersing from biofilms (Robbins et al., 2011). Hsp90 depletion also induces filamentation by relieving Hsp90-mediated repression of the cAMP-PKA signalling pathway. Another protein identified as playing a role in biofilm dispersal is the cell wall protein Ywp1, where deletion of YWP1 results in decreased biofilm dispersal and increased biofilm adhesiveness (Granger, 2012).

The biofilm formation in *Candida albicans* is controlled by a complex network of transcription regulators, as illustrated in Figure 2. The core protein network consists of nine regulators (Ndt80, Bcr1, Rfx2, Flo8, Rob1, Brg1, Gal4, Tec1, and Efg1) that are required for biofilm development. In this network, autoregulation is shown with dashed arrows, direct binding interactions between two regulators that each regulate the activity of the other are shown with dark grey double-headed arrows, and direct binding interactions where one regulator controls another regulator are shown with light grey single-headed arrows (Granger, 2012). This complex regulatory network highlights the intricate molecular mechanisms underlying *Candida albicans* biofilm formation and provides potential targets for therapeutic interventions aimed at disrupting biofilm development in clinical settings (Rodriguez et al., 2020).

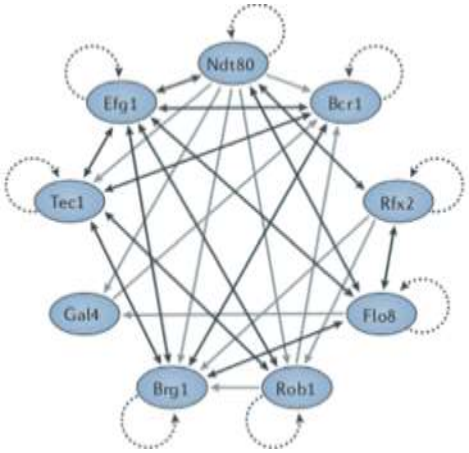

Figure 2. The 50 transcription regulators associated with *Candida albicans* biofilm formation (Rodriguez et al., 2020).

The proteins depicted represent the core network of nine regulators (Ndt80, Bcr1, Rfx2, Flo8, Rob1, Brg1, Gal4, Tec1 and Efg1) required for biofilm development. Autoregulation is shown with dashed arrows, direct binding interactions between two regulators that each regulate the activity of the other are shown with dark grey double-headed arrows, and direct binding interactions where one regulator controls another regulator are shown with light grey single-headed arrows (Granger, 2012).

Factors Influencing Candida Biofilm Formation

Substratum and Surface Properties

The physical and chemical properties of the substratum significantly influence *Candida albicans* biofilm formation. Surface topography plays a crucial role, with biofilm formation being strongly dependent on particle size and surface roughness. Studies using silica particles of varying diameters (0.5-8.0 μm) demonstrate that biofilm formation is significantly enhanced in the 4.0-8.0 μm range compared to smaller particles. Candida species show preferential attachment to hydrophobic surfaces, with most species exhibiting increased biofilm formation on Teflon, except *C. glabrata*, which prefers polyvinyl chloride surfaces. Medical devices are rapidly conditioned by body fluids containing glycoproteins, which alter surface properties and promote biofilm attachment through both non-specific factors (hydrophobicity and electrostatic forces) and specific adhesin-ligand interactions (Atriwal et al., 2021).

Host Factors and Environmental Conditions

Environmental conditions trigger specific genetic responses that modulate biofilm formation through adaptive gene expression programs. Surface properties and host factors activate signal transduction pathways that culminate in transcriptional changes affecting adhesin expression and biofilm development. The response to different substrata involves genetic circuits that sense surface chemistry and topography, leading to differential expression of adhesion-related genes. Host proteins such as fibrinogen and fibronectin not only serve as conditioning films but also trigger genetic responses that enhance adhesin gene expression and biofilm formation capacity. Nutrient availability, pH, and temperature conditions activate stress response pathways

and metabolic gene networks that influence biofilm architecture and resistance properties. These environmental sensing mechanisms demonstrate how genetic regulatory networks integrate external cues to control biofilm development and adaptation (Ng et al., 2016).

Candida-Specific Adhesins and Molecular Mechanisms

Critical biofilm-related adhesins include members of the ALS family, with Als1 and Als3 mediating initial adherence, while Hwp1 functions as a crucial adhesin during hyphal formation. Molecular studies have identified biofilm-associated genes including HWP1 (572 bp) and ALS1 (318 bp) as key determinants of attachment and biofilm formation. The *Candida albicans* cell wall, composed primarily of carbohydrates and glycoproteins, contains β-glucan and chitin forming the inner core, while mannoproteins including adhesins form the outer fibrillar layer that mediates adherence. The filamentation pathway controlled by the Efg1 regulator protein is required for normal biofilm formation and development, with morphogenesis necessary for spatially organized biofilm structures (Gulati & Nobile, 2016).

Quorum Sensing and Cell Communication

Candida albicans produces key quorum sensing molecules including farnesol and tyrosol that regulate morphogenesis and biofilm formation. Farnesol inhibits hyphal formation in a concentration-dependent manner, while tyrosol stimulates the yeast-to-hyphal transition. Biofilm cells secrete at least 50% more tyrosol than planktonic cells, with tyrosol activity being most significant during early and intermediate stages of biofilm development. Farnesol at concentrations of 0.001-3 mM and tyrosol at 1-20 mM demonstrate dose-dependent effects on biofilm formation, with farnesol showing dominant inhibitory effects when both molecules are present. During biofilm development, *Candida albicans* cells within the matrix release these chemical signals that play crucial roles in developing mature biofilm characteristics and coordinating biofilm activities through intercellular communication mechanisms (Atriwal et al., 2021; Rodrigues & Černáková, 2020).

Clinical Significance and Antimicrobial Resistance

Biofilm formation represents a significant clinical risk factor, with biofilm-producing *Candida albicans* isolates associated with increased mortality in candidemia patients. Among clinical isolates, biofilm formation is significantly associated with azole resistance and aspartyl proteinase production, complicating therapeutic interventions. All clinical *Candida parapsilosis* species complex isolates demonstrate biofilm-forming ability, with metabolic activity and biomass production showing significant correlation. Novel therapeutic approaches such as N-acetylcysteine demonstrate inhibitory effects on biofilm formation by down-regulating expression of biofilm-related genes including CpEFG1. These quorum sensing molecules have also demonstrated antifungal and anti-biofilm effects at supraphysiological concentrations, providing potential therapeutic targets (Brunetti et al., 2019; Jakab et al., 2024).

Relationship Between Biofilm Formation and Infectious Disease Pathogenesis

The epidemiological evidence linking Candida biofilms to infectious diseases is substantial, with biofilm formation representing a critical virulence factor in fungal pathogenesis. Candida species are among the most common nosocomial fungal pathogens and are notorious for their propensity toward biofilm formation on medical devices and mucosal surfaces. These infections are associated with high mortality rates of approximately 40-50%, with biofilm-producing Candida isolates significantly associated with increased mortality in candidemia patients. The National Institutes of Health estimates that pathogenic biofilms directly or indirectly cause more than 80% of microbial infections, with virtually all Candida species linked to clinical candidiasis capable of forming highly resistant biofilms on different types of surfaces (Amann et al., 2025; Rajendran et al., 2016).

Candida biofilms demonstrate remarkable resistance to host immune systems through sophisticated evasion mechanisms that significantly impair immune cell function. The extracellular matrix surrounding biofilm cells provides physical protection against immune cell infiltration, while biofilm-associated cells show reduced susceptibility to destruction by neutrophils and macrophages compared to planktonic counterparts. Notably, mature biofilms fail to elicit robust oxidative responses from neutrophils, which represent one of the main pathogen-killing mechanisms. Additionally, monocytes can become embedded within biofilms, inadvertently strengthening the biofilm structure, while macrophage migration is hindered and cytokine responses are altered. The biofilm environment triggers elevated levels of proinflammatory cytokines like IL-1β and MCP-1, but paradoxically also induces IL-10 responses that promote biofilm persistence rather than clearance (Garcia-Perez et al., 2018; Nett et al., 2015).

The clinical significance of Candida biofilm formation is particularly evident in device-associated infections, where biofilms serve as persistent sources of infection and reservoirs for continuing infections. Devices such as stents, shunts, prostheses, implants, endotracheal tubes, pacemakers, and various types of catheters support colonization and biofilm formation by Candida species. Fungi, mainly *Candida albicans*, represent the third leading cause of catheter-related infections, with the second highest colonization-to-infection rate and the overall highest crude mortality. The three-dimensional architecture of mature Candida biofilms, characterized by yeast and hyphal cells embedded within a protective extracellular matrix, facilitates sustained fungal growth while serving as a source for dissemination to distant body sites (Chandra & Mukherjee, 2015; Fan et al., 2022).

Candida biofilms exhibit profound resistance to antifungal agents through multiple synergistic mechanisms that dramatically complicate therapeutic management. The extracellular matrix acts as a diffusion barrier, with drug sequestration by matrix glucan representing a major resistance mechanism. Biofilm cells demonstrate antimicrobial tolerance distinct from resistance, surviving antifungal concentrations more than 1000 times the minimum inhibitory concentrations defined for planktonic cells. Azole antifungals show dramatically reduced efficacy against biofilm-associated cells, while only echinocandins and amphotericin B lipid formulations demonstrate efficacy against established biofilms. The heterogeneous cell populations within

biofilms include metabolically diverse subpopulations and persister cells with dormant-like physiology that contribute to treatment failure (Nicolas et al., 2021).

The dispersal of cells from mature Candida biofilms represents a critical mechanism for disease dissemination and establishment of secondary infection foci throughout the host. Yeast cells released from biofilms exhibit novel properties including increased virulence, enhanced biofilm-forming capability, and altered drug tolerance compared to initial planktonic cells. This dispersal process, occurring through both active and passive mechanisms, enables colonization of new sites and perpetuates the infection cycle. The ability of dispersed biofilm cells to rapidly establish new biofilm communities at distant sites contributes to the recurrent nature of many Candida infections and explains the high failure rates observed with conventional antifungal therapies that target only planktonic cells while leaving biofilm reservoirs intact (Cavalheiro & Teixeira, 2018; Kernien et al., 2018).

Conclusion

The genetic regulation of *Candida albicans* biofilm formation involves complex transcriptional networks that control each developmental stage through specific regulatory genes and pathways. Key transcriptional regulators including Bcr1p, Efg1p, Tec1p, and Rlm1p orchestrate the temporal expression of adhesin genes (Als family, Hwp1), matrix-associated genes (FKS1, glucan transferases), and morphogenesis-related genes throughout the biofilm lifecycle. Quorum sensing molecules such as farnesol and tyrosol function as genetic switches that modulate biofilm-related gene expression in response to cell density and environmental conditions. Understanding these genetic mechanisms provides valuable insights into biofilm biology and offers potential therapeutic targets for developing novel antifungal strategies that can disrupt biofilm formation by interfering with essential regulatory pathways, ultimately improving treatment outcomes for biofilm-associated candidiasis.

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