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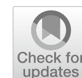
REVIEW



Antifungal properties of cathelicidin LL-37: current knowledge and future research directions

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Abstract

The threat of fungal diseases is substantially underestimated worldwide, but they have serious consequences for humans, animals, and plants. Given the limited number of existing antifungal drugs together with the emergence of drug-resistant strains, many researchers have actively sought alternatives or adjuvants to antimycotics. The best way to tackle these issues is to unearth potential antifungal agents with new modes of action. Antimicrobial peptides are being hailed as a promising source of novel antimicrobials since they exhibit rapid and broad-spectrum microbicidal activities with a reduced likelihood of developing drug resistance. Recent years have witnessed an explosion in knowledge on microbicidal activity of LL-37, the sole human cathelicidin. Herein, we provide a summary of the current understanding about antifungal properties of LL-37, with particular emphasis on its molecular mechanisms. We further illustrate fruitful areas for future research. LL-37 is able to inhibit the growth of clinically and agronomically relevant fungi including *Aspergillus*, *Candida*, *Colletotrichum*, *Fusarium*, *Malassezia*, *Pythium*, and *Trichophyton*. Destruction of the cell wall integrity, membrane permeabilization, induction of oxidative stress, disruption of endoplasmic reticulum homeostasis, formation of autophagy-like structures, alterations in expression of numerous fungal genes, and inhibition of cell cycle progression are the key mechanisms underlying antifungal effects of LL-37. Burgeoning evidence also suggests that LL-37 may act as a potential anti-virulence peptide. It is hoped that this review will not only motivate researchers to conduct more detailed studies in this field, but also inspire further innovations in the design of LL-37-based drugs for the treatment of fungal infections.

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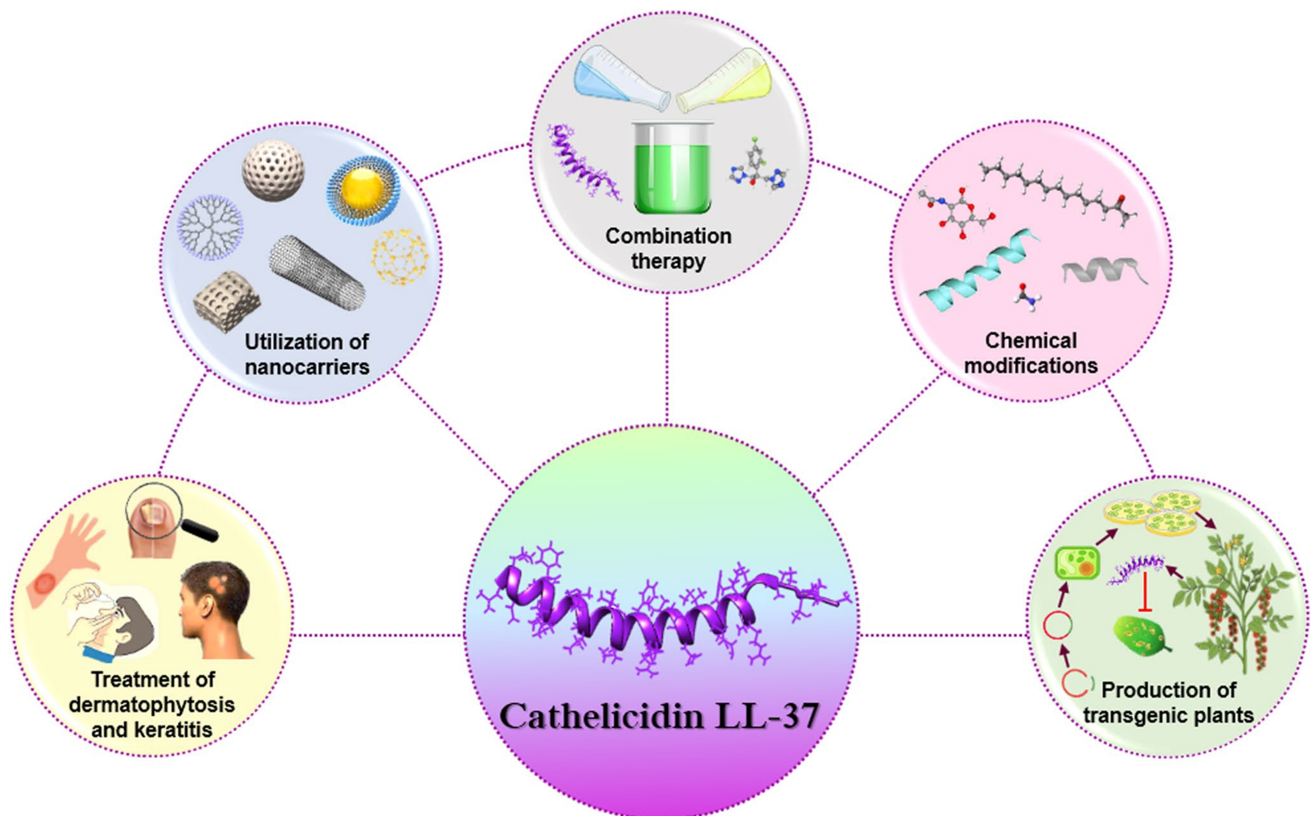
Graphical abstract



Keywords Cathelicidin LL-37 · Antifungal properties · Mechanism of action · Biofilm

Introduction

Fungal infections are responsible for almost 1.6 million deaths, a number equal to that of tuberculosis and three times that of malaria (Bongomin et al. 2017; Alves et al. 2023). The global burden of fungal diseases is expected to rise substantially in the forthcoming years, secondarily owing to the expanding number of individuals living with human immunodeficiency virus (HIV), transplant receivers, immunosuppressive drugs users, patients with advanced cancer, and the elderly (Brackin et al. 2021). Even though the epidemiology of fungal diseases has undergone many changes over the past decades, certain pathogens such as *Aspergillus*, *Candida*, *Cryptococcus*, *Pneumocystis*, and *Histoplasma* are still the principal culprits implicated in the invasive forms of mycoses (Bongomin et al. 2017). Indubitably, the impact of fungi upon living beings goes far beyond causing infectious diseases. The presence of mycotoxins in agricultural products poses either visible, acute effects or chronic, long-term hidden damages to humans and animals (Adedara and Owumi 2023).

As opposed to antibiotics that target specific bacterial structures, the development of antifungal drugs confronts a fundamental problem: similarities between mammalian and fungal cells (Mosallam et al. 2022). In fact, the biochemical pathways and cytoplasmic organelles of fungal cells are analogous to those of human cells because of their eukaryotic heritage. As a result, only a handful of clinically useful classes of antifungal agents have hitherto been developed, such as azoles, allylamines, echinocandins, polyenes, and pyrimidine analogs (Vanreppelen et al. 2023). The long-term therapeutic efficacy of antifungal drugs may be counterbalanced by their limited tolerability and serious adverse effects. To add insult to injury, resistance to antifungals is growing at an unprecedented rate, primarily on account of the unbridled and indiscriminate use of such agents outside of medical care facilities (Memariani et al. 2022). The best way to tackle these issues is to unearth any potential novel antimicrobial agents with new modes of action.

Ubiquitous throughout all biological kingdoms, antimicrobial peptides (AMPs) have transcended million years of evolution and are amongst the most ancient constituents of the immune system (Yarbrough et al. 2015). These peptides

serve as endogenous antibiotics of the host to stave off pathogenic invaders. Aside from variations in their amino acid sequences, AMPs are typically less than 50 residues in length, hydrophobic, and cationic at physiological pH, all of which contribute to their antimicrobial activities (Memariani et al. 2020). Mechanistically, AMPs directly attack microbial membranes and/or counteract intracellular machinery in order to kill or to stymie the proliferation of pathogens (de Souza et al. 2023). In addition, anticancer potential of various AMPs is now beginning to be appreciated (Tornesello et al. 2020). The last two decades have witnessed an explosion of research efforts aiming at developing novel AMP-based drugs since they exhibit rapid and broad-spectrum microbicidal activities with a low probability of drug-resistance evolution in infectious diseases (Datta and Roy 2021; Memariani et al. 2018). Although these endeavors have yielded some success, the full therapeutic potential of AMPs remains untapped.

Cathelicidins are cationic peptides with amphipathic properties that form an integral part of the immune system of many vertebrates, including humans and farm animals (Holani et al. 2023). The cathelicidin family possesses two functional domains, one at the N-terminus which is conserved (the cathelin pro-domain) and another at the C-terminus which is highly variable and exhibits antimicrobial properties. Proteases remove the pro-domain from cathelicidins once they are secreted, forming mature bioactive peptides (Aghazadeh et al. 2019). At the time of writing, there are over 140 amino acid sequences of cathelicidins available in the Antimicrobial Peptide Database (https://aps.unmc.edu).

LL-37 is the only member of the cathelicidin family found in humans to date. An 18 kDa precursor of cationic AMP, hCAP18, is encoded by a gene called *CAMP*. By cleaving hCAP-18 with proteinase-3, LL-37 is released as an active helical peptide of ~ 4.5 kDa (Gwyer Findlay et al. 2013). As the name suggests, LL-37 (LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTES) consists of a total of 37 amino acids; the two leading residues are leucine. Its structure consists of a curved amphipathic helix-bend-helix motif spanning residues 2–31, followed by a disordered C-terminal tail (Wang 2008). The primary source of LL-37 is neutrophils, but it was later shown to be expressed by various cells and tissues such as keratinocytes in inflamed skin, surface epithelial cells of the conducting airways, and mucous cells of the submucosal glands (Wang et al. 2019). LL-37 has well-documented antimicrobial activities toward bacteria, fungi, viruses, and even parasites. It can also exert a multitude of immunomodulatory effects by augmenting cellular killing capacity, differentiating of immune cells, recruiting leukocytes, inducing or mitigating the production of pro-inflammatory cytokines, triggering apoptosis, and promoting angiogenesis (Memariani and Memariani 2023). Although these immunomodulatory effects play a pivotal role in killing microbial cells, their discussion does not fit into the scope of the present review.

LL-37 has so far been extensively investigated for its antimicrobial properties against a gamut of fungal pathogens. Despite the wealth of data that has been amassed on the antifungal effects of LL-37, no attempt has been made to provide a comprehensive review of the literature on this subject. Hence, this review is intended to summarize the current knowledge on the antifungal properties of the human cathelicidin LL-37 and to discuss potential directions for future studies. Particular attention is devoted to the molecular mechanisms underlying antifungal activities of LL-37. For the sake of readers' convenience, each section commences with a brief explanation concerning the significance of the relevant fungal pathogens.

Antifungal effects of LL-37 on planktonic fungal cells

Effects on *Aspergillus* species

Aspergillus species are filamentous fungi that exist as saprobes in soil and vegetative material. The exposure to conidia can lead to a number of clinically significant outcomes, from asymptomatic colonization to invasive infections. Those with structural lung diseases are more likely to develop a fungus ball (aspergilloma) or chronic pulmonary aspergillosis, whereas hypersensitivity to fungal antigens after repeated inhalations of airborne conidia in susceptible hosts may give rise to allergic bronchopulmonary aspergillosis (Russo et al. 2020). Invasive forms of the disease also occur more frequently in patients with severe respiratory viral infections such as influenza, respiratory syncytial virus infection, and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Physiologically, invasive pulmonary forms begin with alveolar damage, and then the fungal pathogen binds to surfactant proteins, leading to epithelial damage and vascular invasion. This is the main reason why invasive pulmonary forms of aspergillosis are deadly and difficult-to-treat diseases. Influenza viruses are responsible for a suppression of NADPH oxidase, causing dysfunction of neutrophils. In SARS-CoV-2, there is a direct damage to the airway epithelium that are thought to enable the invasion by *Aspergillus*. Several risk factors for COVID-associated invasive aspergillosis include age over 62, use of dexamethasone and anti-interleukin-6 (IL-6), and duration of mechanical ventilation exceeding two weeks (Ledoux and Herbrecht 2023). Besides this, glucocorticoids and other immunomodulating drugs may predispose a patient to pulmonary mold infections during treatment for severe SARS-CoV-2 disease (Thompson and Young 2021).

Among the pathogenic aspergilli, *A. fumigatus* is most prevalent in the environment and is responsible for the majority of diseases, followed by *A. flavus*, *A. niger*, *A. terreus*, and *A. nidulans* (Sugui et al. 2015). There exist indications that *A. fumigatus* could upregulate the LL-37 expression levels in human corneal epithelial cells (Zhang et al. 2014) and nasal tissue of chronic rhinosinusitis patients (Ooi et al. 2007), suggesting a conceivable role of LL-37 in the battle against *Aspergillus* infections. These findings have propelled several investigators to address the question of how, and to what extent, the growth and virulence of *A. fumigatus* are affected by LL-37.

In one study (Ballard et al. 2020), a 2-h incubation with LL-37 (5–50 μM) did not lower metabolic activities of clinical and environmental *A. fumigatus* strains (Table 1). In another work (van Eijk et al. 2020), metabolic activity of two azole-resistant *A. fumigatus* strains was not inhibited in the presence of 1 or 5 μM of LL-37. Nevertheless, conspicuous increments in conidial growth of either *A. fumigatus* or *A. flavus* in response to LL-37 treatment were observed by Sheehan et al. (2018). Flow cytometric studies revealed, however, that the average percentage of *A. fumigatus* in the hyphae gate of LL-37-treated cells (12.5 μM) did not differ significantly from that of the control after 10 h, indicating that LL-37 had no inhibitory activity upon *A. fumigatus* hyphal growth (Ballard et al. 2020). Moreover, there were no morphological alterations in hyphae of either *A. fumigatus* or *A. flavus* when challenged with LL-37 (31.25 μg/mL) for 24 h (Sheehan et al. 2018).

Species assigned to the *Aspergillus* genus produce a wide range of mycotoxins, such as aflatoxins, ochratoxins, gliotoxin, fumonisins, sterigmatocystin, and patulin (Abo Nouh et al. 2020). Evidence suggests that LL-37 is capable of enhancing both hyphal mass and gliotoxin secretion (Sheehan et al. 2018). Gliotoxin may play a part in regulation of the redox status of *A. fumigatus* and is believed to protect *A. fumigatus* toward oxidative stress (Gallagher et al. 2012). It would thus seem that cell stress due to the peptide treatment may alter fungal cell redox status, resulting in enhanced production of gliotoxin to restore cell redox homeostasis. Shotgun proteomics of LL-37-treated hyphae also revealed an increase in the abundance of proteins related to growth, tissue degradation, allergic reactions, cellular stress, and virulence (see Table 2). The reader is encouraged to consult the original paper for a more detailed information (Sheehan et al. 2018). These results need further verification in terms of whether or not LL-37 augments expression levels of various mycotoxins and virulence factors in *A. fumigatus*.

Contrary to what was implied above, Luo et al. (2019) demonstrated that LL-37 (1–20 μM) could prevent hyphal growth of *A. fumigatus* in a dose-dependent fashion after 12 h of incubation. The difference in strains may explain these contradictory results. Other factors including the initial inoculum size, the peptide purity, media composition, and the duration of exposure ought to be taken into consideration (Memariani and Memariani 2020). Given that low concentrations of LL-37 may activate compensatory growth pathways in *A. fumigatus*, as discussed by Luo et al. (2019) when comparing their own results to those reported by Sheehan et al. (2018), threshold concentration of LL-37 below which fungal growth occurs should be determined in future research.

So far, only one study has investigated in vitro antifungal activity of LL-37 on *A. nig*er. In this context, Kamysz et al. (2012) found that both LL-37 and its cyclic counterpart exerted an inhibitory activity on *A. nig*er at 128 μg/mL. Complete suppression of the fungal growth was also observed when the concentration of each peptide increased to 512 μg/mL. Additionally, LL-37 and its cyclic analog showed comparable hemolytic activity against the human red blood cells (Kamysz et al. 2012). Antifungal and hemolytic activities of the cyclic LL-37 were identical to those of LL-37. Final judgment on this matter should be withheld until the subject can be followed up by further research.

LL-37 can bind directly to the fungal surface and disrupt the cell wall architecture, leading to inhibition of fungal adhesion in vitro (Table 2). *A. fumigatus* invasion and cytotoxicity on alveolar epithelial cells were weakened in the presence of LL-37 (4 μM), indicating host cell protective activity of LL-37 (Luo et al. 2019). The peptide also reduced the levels of pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) and IL-6, by *A. fumigatus*-stimulated macrophages. Furthermore, LL-37 downregulated transcriptional levels of M1 type markers, such as iNOS, CXCL9, and CXCL10. Neither phagocytosis nor killing capacity of macrophages toward *A. fumigatus* were attenuated by LL-37 (Luo et al. 2019). Based on these results, the conclusion was drawn that the production of *A. fumigatus*-induced pro-inflammatory cytokines by macrophages and expression of M1-type markers could be reduced in response to LL-37 without negatively affecting macrophage phagocytosis or killing activity.

In a preclinical study, wherein mice were intratracheally infected with *A. fumigatus* conidia and then treated with LL-37, lung tissues of the peptide-treated animals had remarkably lower fungal burdens, moderate pathological damage, and reduced levels of pro-inflammatory cytokines (TNF-α and IL-6) as compared to the control (PBS-treated) groups (Luo et al. 2019). A similar trend was observed in transgenic mice expressing hCAP18/LL37. Accordingly, LL-37 may be effective in treating *A. fumigatus* pulmonary infections since it seems to exhibit both anti-inflammatory and antifungal properties. In spite of these promising results, further animal studies are warranted to explore the antimycotic potential of LL-37 against an expanded set of *Aspergillus* strains.

Table 1 Antifungal activities of the human cathelicidin LL-37 against different strains of fungal pathogens

Fungi	Identifiers	Methods	Media for antifungal assays	Antifungal activity	References
Aspergillus flavus	NM	Broth microdilution assay	MMB	• Increasing fungal growth after a 24-h exposure to LL-37 (1.95–250 µg/mL)	Sheehan et al. 2018
Aspergillus fumigatus	V130-15 (isolated from a CGD patient), 111 – 45 (isolated from an AIA patient), and three environmental strains (ENV-S-4, ENV-S-22 and ENV-S-12)	XTT assay	RPMI 1640	• No decrement in metabolic activity of hyphae after a 2-h exposure to 5–50 µM of LL-37	Ballard et al. 2020
	ATCC 26933	Broth microdilution assay	MMB	• Increasing fungal growth after a 24-h exposure to LL-37 (0.97–31.25 µg/mL)	Sheehan et al. 2018
	Two azole-resistant clinical strains (DTO 327-A8 and 326-I1)	Resazurin assay	AMM	• No decrement in metabolic activity of both strains after a 48-h exposure to LL-37 (1 or 5 µM)	van Eijk et al. 2020
Aspergillus niger	NM	Broth microdilution assay	SDB (5%)	• Inhibition of fungal growth at 128 µg/mL after incubation at 25 °C for 48 h	Kamysz et al. 2012
Candida albicans	ATCC 10231	Broth microdilution assay (for LC_{50} determination)	PPB	• LC_{50} value of 0.8 µM after incubation at 37 °C for 1 h	den Hertog et al. 2005
	ATCC 10231	Broth microdilution assay	SDB (5%)	• Inhibition of fungal growth at 128 µg/mL after incubation at 25 °C for 48 h	Kamysz et al. 2012
	ATCC 14053	MFC determination	Dixon's medium (20%)	• MFC value of 20 µM after incubation at 37 °C for 24 h	Murakami et al. 2004
	ATCC 14053	Broth microdilution assay and MFC determination	Dixon's medium (20%)	• Complete inhibition of fungal growth at 25 µM • MFC value of 50 µM after incubation at 25 °C for 24 h	Dorschner et al. 2004
	ATCC 14053	Broth microdilution assay	Dixon's medium (20%)	• MIC value of 16 µM after incubation at 25 °C for 24 h	Braff et al. 2005
	ATCC 14053	Broth microdilution assay	Modified Dixon's medium (20%)	• MIC value of 15 µM after incubation at 25 or 37 °C for 48 h	López-García et al. 2005
	ATCC 90028	RDA	Low-EEO agarose (1%), NaCl (150 mM), and Tween 20 (0.02%) in TSB (0.05%)	• MEC values of 10 and ˃40 µM in low (0 mM) and physiological (150 mM) concentration of NaCl, respectively	Ciornei et al. 2005

Table 1 (continued)

Fungi	Identifiers	Methods	Media for antifungal assays	Antifungal activity	References
	ATCC 90028	RDA	Low-EEO agarose (1%), NaCl (150 mM), and Tween 20 (0.02%) in TSB (0.05%)	• Inhibition of fungal growth at different concentrations (20–100 µM)	Sigurdardottir et al. 2006
	ATCC 90028 and three clinical strains (obtained from patients with AD)	RDA and broth microdilution assay (for LC_{50} determination)	TSB (1.5 mg), Low-EEO agarose (50 mg), and Tween 20 (0.02%) dissolved in 10 mM Tris, pH 7.4	• DCZ of ~ 2.5–3.2 mm for LL-37 (50 or 100 µM) against *C. albicans* ATCC 90028 and two clinical strains • LC_{50} value of 11.5 µM	Sonesson et al. 2007
	SC5134	XTT assay	RPMI 1640	• Remarkable reduction in metabolic activity of yeast cells after a 24-h exposure to LL-37 (0.5–10 µM)	Wong et al. 2011
	SC5314	Spot assay (for evaluation of cell viability) and FUN-1 assay (using flow cytometry)	RPMI 1640	• Remarkable reduction in yeast cell viability after exposure to ≥ 20 µg/mL of LL-37 for 30 min • Killing around 60% of yeast cells after a 30-min incubation with 40 µg/mL of LL-37	Tsai et al. 2011a
	SC5314	Broth microdilution assay (colony counting for evaluation of cell viability)	PBS	• Candidacidal activity of LL-37 (˃10 µM) at 30 °C after 30 min	Chang et al. 2012
	SC5314	Spot assay (for evaluation of cell viability)	RPMI 1640	• Dose-dependent reduction in yeast cell viability • LC_{50} value of 30 µg/mL (~ 6.6 µM) after incubation at 37 °C for 30 min	Tsai et al. 2014
	SC5314 and twelve azole-susceptible strains (obtained from patients with vaginitis)	Broth microdilution assay	SDB	• MIC range of 16 – ≥64 µM after incubation at 30 °C for 48 h	Scarsini et al. 2015
	ATCC 10231	MFC determination	HEPES buffer	• LL-37 had a slightly higher MFC of 10 µM after incubation at 37 °C for 3 h	Ordonez et al. 2014
	ATCC 10231	Broth microdilution assay	RPMI 1640	• No growth-inhibitory activity at 28.5 µM	Lima et al. 2017
	ATCC 2002 and seven clinical strains	Broth microdilution assay	RPMI 1640	• MIC values of 16–32 µg/mL after incubation of the standard stain with LL-37 at 35 °C for 48 h • No apparent antifungal activity against the clinical strains	Yu et al. 2016a

Table 1 (continued)

Fungi	Identifiers	Methods	Media for antifungal assays	Antifungal activity	References
	Three strains (CA 1407, CA 1408 and CA 1409, from the PAN, Wroclaw, Polish Collection of Microorganisms)	Broth microdilution assay and MFC determination	PBS	• MIC/MFC values of 64/128, $>$ 256/$>$ 256, and $>$ 256/$>$ 256 µg/mL for CA 1407, CA 1408 and CA1409, respectively, after a 1-h incubation with LL-37 at 37 °C	Durnaś et al. 2016
Candida auris	Ten clinical strains	Broth microdilution assay and MFC determination	RPMI 1640	• MIC range of 25–100 µg/mL after incubation at 37 °C for 24 h • MFC range of 50–200 µg/mL	Rather et al. 2022
Candida glabrata	Seven clinical strains obtained from patients with vaginitis (6/7 SDD and 1/7 R to ITC; 2/7 SDD to FLC)	Broth microdilution assay	SDB	• MIC values of > 64 µM after incubation at 30 °C for 48 h	Scarsini et al. 2015
Candida krusei	Three clinical strains obtained from patients with vaginitis (3/3 SDD to ITC; 2/3 SDD and 1/3 R to FLC)	Broth microdilution assay	SDB	• MIC range of 4–64 µM after incubation at 30 °C for 48 h	Scarsini et al. 2015
Candida norvegensis	A clinical strain obtained from patient with vaginitis (SDD to ITC and to FLC)	Broth microdilution assay	SDB	• MIC value of 16 µM after incubation at 30 °C for 48 h	Scarsini et al. 2015
Candida parapsilosis	ATCC 90018	RDA	Low-EEO agarose (1%), NaCl (150 mM), and Tween 20 (0.02%) in TSB (0.05%)	• Inhibition of fungal growth at different concentrations (10–40 µM)	Sigurdardottir et al. 2006
	An azole-susceptible strain obtained from a patient with vaginitis	Broth microdilution assay	SDB	• MIC value of 32 µM after incubation at 30 °C for 48 h	Scarsini et al. 2015
Candida tropicalis	NM	Broth microdilution assay	SDB (5%)	• Inhibition of fungal growth at 128 µg/mL after incubation at 25 °C for 48 h	Kamysz et al. 2012
Fusarium graminearum	Wheat isolate CS3005	Spectroscopic analysis of fungal growth inhibition	Half-strength PDB	• IC_{50} value of 1 µM after incubation at 25 °C for 24 h	van der Weerden et al. 2010
Fusarium oxysporum f. sp. *vasinfectum*	Australian isolate VCG01111	Spectroscopic analysis of fungal growth inhibition and MTT assay	Half-strength PDB	• IC_{50} value of 2.5 µM after incubation at 25 °C for 24 h • Reduction in hyphal viability at peptide concentrations of 2.5, 5, and 10 µM	van der Weerden et al. 2010
Malassezia furfur	ATCC 46267	Broth microdilution assay	Modified Dixon's medium (20%) or PDB	• Complete inhibition of fungal growth at 25 µM (MIC of 20–30 µM) after incubation at 30 °C for 24 h	López-García et al. 2006

Table 1 (continued)

Fungi	Identifiers	Methods	Media for antifungal assays	Antifungal activity	References
Microsporum canis	CECT 2797	Broth microdilution assay and MFC determination	Modified Dixon's medium (20%) or PDB	• MIC and MFC values of ˃ 100 μM after incubation at 25 °C for 5 days	López-García et al. 2006
Pythium insidiosum	Eleven strains recovered from equine pythiosis and three standard strains (CBS 77784, CBS 57585, and CBS 119455)	Broth microdilution assay	RPMI 1640-MOPS	• MIC range and mean of 20–40 μM and 29.71 μg/mL, respectively	Denardi et al. 2022
Saccharomyces cerevisiae	Three azole-susceptible strains (obtained from patients with vaginitis)	Broth microdilution assay	SDB	• MIC value of 2 μM for all strains after incubation at 30 °C for 48 h	Scarsini et al. 2015
Trichophyton mentagrophytes	var. *goetzii* CECT 2957 and var. *interdigitale* CECT 2958	Broth microdilution assay and MFC determination	Modified Dixon's medium (20%) or PDB	• MIC and MFC values of 12.5 μM after incubation at 25 °C for 5 days	López-García et al. 2006
Trichophyton rubrum	CECT 2794 and a clinical strain	Broth microdilution assay and MFC determination	Modified Dixon's medium (20%) or PDB	• MIC and MFC values of 12.5 and 25 μM, respectively, after incubation at 25 °C for 5 days	López-García et al. 2006

AD Atopic dermatitis; *AIA* Acute invasive aspergillosis; *AMM* Aspergillus minimal medium; *CGD* Chronic granulomatous disease; *DCZ* Diameter of the clear zone; *FLC* Fluconazole; *HEPES* 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; IC_{50} Half-maximal inhibitory concentration; *ITC* Itraconazole LC_{50} The concentration causing 50% reduction of viable cells; *Low-EEO* Low-electroendosmosis type; *MEC* Minimum effective concentration; *MFC* Minimum fungicidal concentrations; *MIC* Minimum inhibitory concentration; *MMB* Minimal medium broth (containing 2% glucose and 0.5% yeast nitrogen base); *MOPS* 3-(*N*-morpholino)propanesulfonic acid; *MTT* 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; *NM* Not mentioned; OD_{570nm} Optical density at 570 nm; *PBS* Phosphate-buffered saline; *PDB* Potato dextrose broth; *PPB* Potassium phosphate buffer; *R* Resistant; *RDA* Radial diffusion assay; *RPMI* Roswell Park Memorial Institute; *SDB* Sabouraud's dextrose broth; *SDD* Susceptible-dose dependent; *TSB* Trypticase soy broth; *XTT* 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide

Table 2 Antifungal mechanisms of action of the human cathelicidin LL-37 against different fungi

Fungi	Identifiers	Methods	Key findings	References
Aspergillus flavus	NM	Fluorescence microscopy	• No effect on hyphal morphology	Sheehan et al. 2018
Aspergillus fumigatus	Strain AF293	Adhesion assay, TEM, flow cytometry, confocal microscopy, hyphal growth inhibition assay, LDH release assay, and phagocytosis assay	• Direct binding of LL-37 to the surface of resting conidia • Disruption of the cell wall integrity • Inhibition of hyphal growth in a dose-dependent manner • Inhibition of mycelial adhesion to microplates and subsequent biofilm formation • Reduction of *A. fumigatus* invasion and toxicity on alveolar epithelial cells • Reduction of *A. fumigatus*-induced TNF-α and IL-6 production by BMDMs • Downregulation of mRNA levels of iNOS, CXCL9, and CXCL10 • No attenuation of either phagocytosis or killing capacity of macrophages against *A. fumigatus* in the presence of LL-37	Luo et al. 2019
	V130-15 (isolated from a CGD patient), 113 – 61 (isolated from a CF patients), 151-06 (isolated from a CPA patient), 120 – 76 (isolated from an AIA patient), and ENV-S-22 (an environmental strain)	Flow cytometry	• No activity on hyphal growth	Ballard et al. 2020
	ATCC 26933	Fluorescence microscopy, determination of hyphal wet weight, quantification of gliotoxin by RP-HPLC, and shotgun proteomics	• No effect on hyphal morphology • Increasing hyphal wet weight • Increasing gliotoxin secretion from peptide-treated hyphae • Increasing relative abundance of different proteins as compared to the control: eIF-5A (16.3-fold), aspartic endopeptidase (4.7-fold), Asp F13 (10-fold), glutathione peroxidase (9-fold), and RNase mitogillin (3.7-fold)	Sheehan et al. 2018
Candida albicans	ATCC 10231	Killing kinetics (by measuring PI fluorescence), IEM, FFEM, determination of nucleotides in *C. albicans* supernatants, and SDS-PAGE	• Rapid fungicidal activity (an immediate increase in PI fluorescence; within 5 min) • Localization of the majority of LL-37 at the cell wall and cell membrane • Disintegration of the membrane bilayer into discrete vesicles • Inducing leakage of nucleotides and proteins (originated from cytoplasm, plasma membrane, and cell wall)	den Hertog et al. 2005; den Hertog et al. 2006

Table 2 (continued)

Fungi	Identifiers	Methods	Key findings	References
	ATCC 14053	Killing kinetics and membrane permeabilization assay (uptake of SG)	• Effective fungicidal activity of LL-37 (25 μM) at inoculum density of $2.5–5 \times 10^4$ CFUs/mL, but not 10^6 CFUs/mL • Rapid permeabilization of fungal membrane (˃50% after a 5-min incubation) • Correlation between kinetics of permeabilization and fungicidal effects of LL-37	López-García et al. 2005
	ATCC 90028	SEM	• Significant perturbations of the cell surface after a 2-h incubation with either 50 or 100 μM of LL-37	Sonesson et al. 2007
	SC5314	SEM (for monitoring hyphal growth)	• Slenderizing the hyphal thickness • Reduction in budding growth and cell viability	Wong et al. 2011
	SC5314	Adhesion assay (XTT assay and ELISA), light microscopy, SDS-PAGE, Western blotting, flow cytometry, and evaluation of LL-37 binding to polysaccharides	• Inhibition of yeast cell adhesion to polystyrene in a dose-dependent manner • Inducing aggregation of yeast cells in a dose-dependent manner • Binding of LL-37 to floating yeast cells • Interaction with yeast cell wall carbohydrates such as mannan, chitin, and glucan • Inhibition of yeast cell adhesion to oral epidermal cells and mouse bladder mucosa	Tsai et al. 2011a
	SC5314 and different mutant strains	Flow cytometry, extraction of CWPs, SDS-PAGE, Western blotting, phage-display biopanning, ELISA, DNA sequencing, searching for the potential LL-37 interacting proteins from *Candida* Genome Database, and adhesion assay (using XTT)	• Inhibition of LL-37 binding to *C. albicans* after removing CWPs using proteinase K • Binding of LL-37 to three CWPs • Interactions between LL-37 and 10 of the phage-displayed peptides (all contained the consensus sequence of ΦHWXΦΦXΦXΦ in which Φ is a hydrophobic residue and X represents any residue) • High similarity of four peptide sequences in the major *C. albicans* cell wall β-1,3-exoglucanase (Xog1p) to the above-mentioned consensus sequence • Binding of LL-37 to $Xog1p_{90–115}$ in a dose-dependent and saturable manner • Reversing the anti-adhesive activity of LL-37 on *C. albicans* by adding $Xog1p_{90–115}$ • Reduction of cellular exoglucanase activity, cell adhesion, and LL-37 binding to yeast cells in mutants with deletion of *XOG1*	Tsai et al. 2011b

Table 2 (continued)

Fungi	Identifiers	Methods	Key findings	References
	SC5314	Adhesion assay (colony counting), pull-down assays, and ELISA	• Inhibition of yeast cell adhesion to plastic after a 30-min incubation with either 3 or 10 µM of LL-37 • Interactions between LL-37 and Xog1p(41–438)-6 H and Xog1p fragments • Increasing *β*-1,3-exoglucanase activity of Xog1p(41–438)-6 H (1.8-fold increase using 1 µM of LL-37 as compared with the control)	Chang et al. 2012
	SC5314	TEM, measurement of the cell wall carbohydrate contents, flow cytometry, DNA microarray analysis, and quantitative real-time PCR	• Reduction of *C. albicans* cell wall thickness (~30%) and cell wall polysaccharides (glucan, ~25%; and mannan, ~30%) using LL-37 (20 µg/mL) as compared to the control • Disrupting the process of cell wall reconstruction, resulting in the exposure of cell wall β-1,3-glucan • Substantial changes in expression levels of 83 genes (affecting different biological processes)	Tsai et al. 2014
	ATCC 10231	ATP release assay, confocal live-cell imaging of LL-37-induced killing of *C. albicans*, flow cytometry, and TEM	• Inducing ATP release at 5 and 60 min after contact with LL-37 • Rapid localization of FITC-labeled LL-37 to the *C. albicans* cell membrane • Triggering PI influx and vacuolar expansion at around 450 s using 10 µM (MFC) of LL-37 • No decrease in cell size after incubation with MFC of LL-37 • Energy depletion by sodium azide did not affect the activity of LL-37 • Inducing detachment of the plasma membrane from the cell wall	Ordonez et al. 2014
	CA 1408 (obtained from the PAN, Wroclaw, Polish collection of microorganisms)	Fluorescence spectroscopy, SEM, and AFM	• Affinity of FITC-labeled LL-37 to fungal membrane • Induction of morpho-structural deformities in a dose-dependent manner • Formation of cell surface wrinkles • Induction of small, crack-like break in the cell surface • Alteration of cell shape from oval to more elongated forms	Durnaś et al. 2016

Table 2 (continued)

Fungi	Identifiers	Methods	Key findings	References
	SC5314	TEM	• Formation of membrane ruptures and membrane internalizations (lollipops) • Formation of autophagy-related structures (such as condensed, yet fragmented nuclear fractions)	Menzel et al. 2017
	SC5314 and several mutant strains (such as *sfp1*Δ/*sfp1*Δ)	Flow cytometry, Western blotting, assay for *HAC1* mRNA splicing, quantitative real-time PCR, measurement of intracellular ROS, lipid peroxidation assay (measurement of MDA levels), and evaluation of protein secretion	• Lower percentages of PI-positive cells in the *sfp1*Δ/*sfp1*Δ mutant in comparison to the wild-type and *SFP1*-reintegrated strains after LL-37 treatment • Increasing p-Mkc1 levels in the wild-type strain in response to LL-37 treatment (elevated p-Mkc1 levels were seen in the *sfp1*Δ/*sfp1*Δ mutant; treated with or without LL-37) • Association of *C. albicans* cell response to LL-37 with the impact of *SFP-1* deletion on cell wall integrity • LL-37 activated the UPR, which is indicated by the elevated levels of spliced *HAC1* mRNA in the wild-type (*HAC1* mRNA was poorly spliced in the *sfp1*Δ/*sfp1*Δ mutant; treated with or without LL-37) • *SFP1* deletion attenuated *HAC1* mRNA splicing and UPR-responsive gene activation upon LL-37 treatment • Induction of ER stress, ROS accumulation, and lipid peroxidation upon LL-37 treatment • Significant increases in the levels of total secreted protein were detected in the wild-type and *SFP1*-reintegrated strains (but not in the *sfp1*Δ/*sfp1*Δ mutant) after LL-37 treatment compared to those without treatment	Hsu et al. 2021

Table 2 (continued)

Fungi	Identifiers	Methods	Key findings	References
Candida auris	An MDR strain (resistant to AmB, CAS, and FLZ)	Killing kinetics, antioxidant assays, assessment of cell cycle arrest, fluorescence spectroscopy, and SEM	• Complete killing of *C. auris* cells within 8 h at MFC of LL-37 • Modulation of the activity of antioxidant enzymes (Increasing the activity of CAT, SOD, and GPx as well as decreasing the activity of GLR and GST) • Increasing lipid peroxidation • Induction of cell cycle arrest at S phase • Permeabilization of plasma membrane • LL-37-treated cells had various sizes, irregular shapes, and depressed surfaces	Rather et al. 2022
Fusarium oxysporum f. sp. *vasinfectum*	Australian isolate VCG01111	Kinetics of hyphal membrane permeabilization (quantification of SG uptake)	• Permeabilization of hyphal membrane (the uptake reached 75% and the maximum within 20 and 40 min, respectively)	van der Weerden et al. 2010

AFM Atomic force microscopy; *AIA* Acute invasive aspergillosis; *AmB* Amphotericin B; *BMDMs* Bone marrow-derived macrophages; *CAS* Caspofungin; *CAT* Catalase; *CF* Cystic fibrosis; *CGD* Chronic granulomatous disease; *CPA* Chronic pulmonary aspergillosis; *CWPs* Cell wall proteins; eIF-5A Eukaryotic translation initiation factor 5A; *ELISA* Enzyme-linked immunosorbent assay; *ER* Endoplasmic reticulum; *FFEM* Freeze-fracture electron microscopy; *FITC* Fluorescein isothiocyanate; *FLZ* Fluconazole; *GLR* Glutathione reductase; *GPx* Glutathione peroxidase; *GST* Glutathione transferase; *IEM* Immunoelectron microscopy; *IL*-6 Interleukin-6; *iNOS* Inducible nitric oxide synthase; *LDH* Lactate dehydrogenase; *NM* Not mentioned; *MDA* Malondialdehyde; *MDR* Multi-drug resistant; *MFC* Minimum fungicidal concentrations; *PI* Propidium iodide; *p-Mkc*1 Phosphorylated Mkc1; *ROS* Reactive oxygen species; *RP-HPLC* Reversed-phase high-performance liquid chromatography; *SDS-PAGE* Sodium dodecyl sulphate polyacrylamide gel electrophoresis; *SEM* Scanning electron microscopy; *SG* SYTOX Green; *SOD* Superoxide dismutase; *TEM* Transmission electron microscopy; *TNF-α* Tumor necrosis factor-alpha; *UPR* Unfolded protein response; *XTT* 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2 H-tetrazolium-5-carboxanilide

Effects on *Candida* species

Candida species belong to the normal microbiota of the oral cavity, gastrointestinal tract, and vagina. In the United States, *Candida* species are the fourth leading cause of nosocomial bloodstream infections, with mortality rates reached as high as 40% (Atiencia-Carrera et al. 2022). *C. albicans* is the most important species, a pathobiont that can become pathogenic under certain circumstances. It possesses several virulence attributes, including yeast-to-hypha transition, hydrolytic enzyme secretion, tissue adhesion, and biofilm formation. The emergence of multi-drug resistant *Candida* species, such as *C. glabrata* and *C. auris*, has sparked a great deal of research into antifungal alternatives (Arendrup and Patterson 2017). In the current situation, AMPs may offer a fruitful avenue for developing new anticandidal drugs (Memariani et al. 2023).

There is now a rather significant and consistent body of data on anticandidal activities of LL-37 under different experimental conditions. In the case of *C. albicans*, concentrations ranging from 0.8 to 64 µM were sufficient for both growth-inhibiting and killing actions of LL-37 (Table 1). According to Wong et al. (2011), LL-37 (0.5–12 µM) exhibited similar or perhaps slightly greater anticandidal activity compared to its fragments, namely LL13–37 and LL17–32. LL-37 cleavage into shorter processed forms (such as RK-31 and KS-30) has been shown to confer additional fungicidal activity in ionic environments mimicking sweat (López-García et al. 2005). Based on analyses of truncated variants of LL-37, the stretches of 1–12 and 26–37 do not appear to have substantial anticandidal activity. It has been suggested that overall structural properties of LL-37, such as peptide length, are also important for antifungal potency, even in the absence of an obvious candidacidal domain (den Hertog et al. 2006).

C. albicans cells were reported to be killed by LL-37 in a dose- and pH-dependent manner (Tsai et al. 2014; López-García et al. 2005). Furthermore, the candidacidal activity of LL-37 is unaffected by the metabolic inhibitor sodium azide; thus, this activity is independent of cellular energy status (Ordonez et al. 2014). Nonetheless, LL-37 was considerably less active in vitro when exposed to physiological salt concentrations (Ciornei et al. 2005). One study found that human body fluids (pus, saliva, and urine) decreased anticandidal activity of LL-37, whereas 50% of blood plasma completely abolished its activity (Durnaś et al. 2016).

Regarding localization of LL-37 in *C. albicans*, microscopic studies unraveled that fluorescein isothiocyanate (FITC)-labelled LL-37 remained connected to the cell perimeter (den Hertog et al. 2005; Ordonez et al. 2014). It appears that LL-37 is localized at the cell wall and cell membrane. As judged by freeze-fracture electron microscopy, untreated yeast cells showed a homogenous distribution of intramembraneous particles (IMPs), which are transmembrane proteins. Additionally, these untreated cells showed trough-shaped invaginations that are typical of freeze-fractured yeast cells. A sub-lethal concentration of LL-37 (0.5 µM) led to formation of shallow craters and IMP-free patches in yeast membranes, while increasing the concentration to 2 µM resulted in developing of discrete vesicle-like structures in the membrane leaflets (den Hertog et al. 2006). From these data, it can be concluded that sub-lethal concentrations of LL-37 induce morphological changes in the yeast membrane, while higher concentrations of the peptide are more destructive to the membrane, leading to weakening of the membrane leaflets.

LL-37 is capable of inducing major morpho-structural deformities such as surface wrinkling, cell elongation, and crack-like break formations in yeast cells (Durnaś et al. 2016). Experimental evidence also points to a correlation between membrane-permeabilizing activity of LL-37 and its candidacidal kinetics (López-García et al. 2005). In support of this, confocal live-cell imaging of *C. albicans* cells divulged that LL-37 at its minimum fungicidal concentration (MFC) could induce propidium iodide influx and vacuolar expansion (Ordonez et al. 2014). Rapid permeabilization and perturbation of *C. albicans* membrane, instantaneous release of vital cellular components such as nucleotides and proteins (with molecular masses of <40 kDa), detachment of the plasma membrane from the cell wall, and disintegration of the membrane bilayer into discrete vesicles by LL-37 would eventuate in yeast cell demise (López-García et al. 2005; den Hertog et al. 2006; Sonesson et al. 2007; Ordonez et al. 2014). Collectively, it appears that membrane disruption is an important mechanism by which LL-37 extirpates yeast cells.

There is also some indication that LL-37 may incur damage to the internal cell architecture in *C. albicans*. For example, transmission electron microscope studies of LL-37-treated yeasts revealed round electron-dense structures and mitochondrial distortion, reminiscent of what is observed during autophagy (Menzel et al. 2017). Condensed, yet fragmented nuclear fractions and membrane internalization were also detected in the peptide-treated yeast cells. However, there is still a great deal of mystery surrounding the molecular details underlying such effects.

Adhesion to both biotic and abiotic surfaces is crucial for *C. albicans* pathogenesis since it contributes to fungal persistence, filamentation, and biofilm formation (Martin et al. 2021). Consequently, targeting *C. albicans* adhesion offers a promising strategy for disease prevention. A pair of articles published in 2011 demonstrated that LL-37 could inhibit the adhesion of *C. albicans* (Tsai et al. 2011a, b). In the first study, LL-37 dose-dependently inhibited *C. albicans* attachment to polystyrene by binding directly to the cell surface and evoking cell aggregation. Moreover, sub-lethal

concentrations of LL-37 decreased yeast cell adhesion not only to oral epidermal cells but also to murine bladder mucosa. Interaction of LL-37 with cell wall polysaccharides (such as mannan, chitin, and glucan) may explain the decreased adhesion of yeast cells (Tsai et al. 2011a). Cationic AMPs like LL-37 can interact electrostatically with negatively charged membrane carbohydrates (Chang et al. 2012). Besides carbohydrates, proteins are major components of the *C. albicans* cell wall (Chaffin 2008). In a later work, LL-37 was shown to interact with cell wall proteins (Tsai et al. 2011b). Upon closer inspection, it became clear that LL-37 targets cell wall β-1,3-exoglucanase Xog1p, which is involved in *C. albicans* cell adhesion (Table 1). In fact, LL-37 affects Xog1p activity, thereby impairing cell wall remodeling. It was also suggested that deletion of *XOG1* reduced cellular exoglucanase activity, cell adhesion to polystyrene wells, and LL-37 binding to mutant *C. albicans* cells (Tsai et al. 2011b). In a related study, non-lethal concentrations of LL-37 precluded *C. albicans* adhesion to plastic by enhancing the β-1,3-exoglucanase activity of Xog1p (Chang et al. 2012). Increased β-1,3-exoglucanase activity could lead to abnormal cell wall glucan metabolism, resulting in the suppression of fungal adhesion without inducing perceptible changes in cell wall morphology (Chang et al. 2012).

Mechanistic studies provided additional insight into the effects of LL-37 on *C. albicans* cell wall and its cellular responses (see Table 1). Recently, Hsu et al. (2021) observed that LL-37 could induce cell wall stress in *C. albicans*. In a separate study, LL-37 (20 µg/mL) diminished both *C. albicans* cell wall thickness and total polysaccharide content (i.e. glucan and mannan, but not chitin), suggesting that LL-37 alters cell wall architecture (Tsai et al. 2014). In *C. albicans*, the majority of β-1,3-glucan is located in the inner cell wall and is masked by an outer layer of mannan fibrils (Childers et al. 2020). Yeasts treated with LL-37 (≥ 5 µg/mL) showed substantially more β-1,3-glucan exposed on the cell surface than the control, connoting that the peptide might disrupt cell wall reconstruction.

LL-37 was reported to activate the mitogen-activated protein (MAP) kinase Mkc1 signaling pathway (Hsu et al. 2021). The MAP kinase Mkc1 participates in cell wall integrity of *C. albicans* (Ernst and Pla 2011). LL-37 can also stimulate the unfolded protein response (UPR) pathway to adapt and respond to endoplasmic reticulum (ER) stress conditions. The UPR is the mechanism through which cells control ER protein homeostasis (Read and Schröder 2021). Moreover, LL-37 can trigger ER-derived reactive oxygen species (ROS) accumulation to cause oxidative stress and to influence ER-related protein secretion (Hsu et al. 2021). ER-derived ROS are thought to be involved in toxicity of cell wall stress. Thus, there is a possible link between cell wall integrity, ER function, and ER-derived ROS production in fungal cells (Yu et al. 2016b). Another point worth highlighting is that deletion of the transcription factor *SFP1* reduced the vulnerability of *C. albicans* to LL-37. Experiments on mutants lacking *SFP1* gene also suggest that this transcription factor plays a key role in cell wall maintenance, ER homeostasis, and oxidative stress response upon LL-37 treatment (Hsu et al. 2021).

Genome-wide analysis of LL-37-treated *C. albicans* cells made it clear that exposure to LL-37 (5 µg/mL) caused significant changes in the expression levels of 83 genes (> 1.5-fold change, $p < 0.05$), of which 59 were downregulated while the remainders were upregulated. Based on *C. albicans* genome annotation, these genes influence a wide gamut of biological processes including, but not limited to, RNA metabolism, ribosome biogenesis, cell cycle, cell wall organization, stress responses, and filamentous growth (Tsai et al. 2014). For instance, treatment of *C. albicans* with LL-37 caused upregulation of *NRG1*, *KRE6*, and *TPO3*. The product of *NRG1* was suggested to play a part in the regulation of stress responses, yeast-to-hypha morphological switch, and biofilm establishment (Murad et al. 2001; Tsai et al. 2014). *KRE6* contributes to the synthesis of β-1,6-glucan (Han et al. 2019). *TPO3* encodes a polyamine transporter, required for yeast resistance to the polyamine spermine toxicity (Fernandes et al. 2005). On the other hand, expression levels of *HGT12*, *GAP2, RHR2*, and *TRY4* were shown to be downregulated in response to LL-37 treatment (Tsai et al. 2014). *HGT12* encodes a protein that transports fructose, mannose, and glucose specifically during macrophage infection (Luo et al. 2007). *GAP2* encodes a general amino acid permease (Kraidlova et al. 2016). *RHR2*, which encodes the enzyme glycerol-3-phosphatase, is required for biofilm production in vitro and in vivo (Desai et al. 2013). *TRY4* is needed for yeast cell adherence (Finkel et al. 2012).

The phenotypic plasticity between the two morphological phenotypes, yeast and hyphae, allows *C. albicans* cells to adapt to and persist in different environments. Indeed, the yeast-to-hyphae transition is essential for tissue invasion, escape from phagocytes, and biofilm formation (Wooten et al. 2021; Kong and Jabra-Rizk 2015). According to Wong et al. (2011), *C. albicans* morphogenesis was affected by LL-37 or its fragment (LL13–37), as hyphae became thinner and budding appeared less robust. The exact mechanisms behind these observations need to be elucidated in future studies.

Aside from *C. albicans*, other species of *Candida* were reported to be susceptible to LL-37 treatment. These include *C. auris*, *C. krusei*, *C. norvegensis*, *C. parapsilosis*, and *C. tropicalis* (Kamysz et al. 2012; Rather et al. 2022; Scarsini et al. 2015; Sigurdardottir et al. 2006). According to a recent study, LL-37 exhibited fungicidal activity against 10 clinical isolates of *C. auris*, some of which were resistant to conventional antimycotics (Table 1). Kinetics studies also

suggest that 8-h exposure to MFC of LL-37 was sufficient for complete killing of an MDR *C. auris* strain (Rather et al. 2022). Moreover, LL-37 was shown to permeabilize plasma membrane, arrest cell cycle at S phase, and induce oxidative stress in *C. auris* (Table 1), indicating multifaceted mechanisms of action of LL-37. When combined with fluconazole, amphotericin B, and caspofungin individually, LL-37 showed synergistic effects against 80%, 100%, and 100% of the above-mentioned *C. auris* strains, respectively (Rather et al. 2022). Therefore, LL-37 in combination with the current antifungal drugs could be suggested as a possible treatment for *C. auris* infections.

Nanoparticles might serve as both AMP releasers and protection agents (Maximiano et al. 2022). In this regard, Niemirowicz et al. (2017) observed that immobilization of LL-37 on the surface of magnetic nanoparticles (MNPs) substantially increased fungicidal activity of LL-37 toward laboratory and clinical strains of *C. albicans*, *C. glabrata*, and *C. tropicalis* in the presence of body fluids (e.g., saliva, urine, plasma, pus, and cerebrospinal fluid). They also exhibited low cytotoxicity to human osteoblast cells. These functionalized MNPs disrupted the integrity of fungal plasma membrane and triggered ROS generation, resulting in cell death (Niemirowicz et al. 2017). It appears that high antifungal activity and biocompatibility of these LL-37-coated MNPs make them attractive candidates for the treatment of *Candida* infections.

Effects on *Colletotrichum higginsianum*

Species of the genus *Colletotrichum* cause devastating anthracnose diseases in various agricultural and horticultural crops (da Silva et al. 2020). *C. higginsianum* is one of the most prominent *Colletotrichum* species. In one study, Jung and coworkers (2012) demonstrated transgenic expression of LL-37 with Met37Leu substitution in Chinese cabbage, an important vegetable crop in Asia. The leaves of the transgenic plants were then exposed to *Colletotrichum higginsianum* KACC 40807 to examine whether LL-37 expression could confer them resistance to this fungal pathogen. Transgenic plants exhibited higher resistance to *C. higginsianum* than the non-transgenic control plants, as the average size of disease lesions decreased in the transgenic plants (Jung et al. 2012). In light of the adverse effects that fungi have on many economically important crops, transgenic strategies such as constitutive expression of AMPs could be useful in enhancing plant resistance to phytopathogens.

Effects on dermatophytes

Dermatophytes are a group of closely related keratinophilic fungi that invade keratinous tissues. Infections caused by these pathogens (tinea or ring-worm) usually tend to involve the hair, nail, or skin of the host. Dermatophytes can be classified in three genera, namely *Epidermophyton*, *Microsporum*, and *Trichophyton* (Aref et al. 2022). Some dermatophytes have been reported to be susceptible to LL-37 (López-García et al. 2006). For instance, LL-37 was found to be active as fungicide against different strains of *Trichophyton mentagrophytes* and *Trichophyton rubrum*, but not *Microsporum canis* CECT 2797 in vitro (see Table 1). Based on immunohistochemical studies, elevated LL-37 expression was found in the epidermis of patients with tinea corporis or tinea versicolor in comparison to healthy human skin. Besides this, LL-37 expression was co-localized with the fungus in the stratum corneum. Exposure to *T. rubrum* for 40 h also induced the mRNA expression of LL-37 in cultured normal human keratinocytes (NHKs) (López-García et al. 2006). This is not surprising since, as mentioned earlier, keratinocytes are the most abundant cells in the epidermis and play a crucial role in the initial response to dermatophytes. In fact, these cells secret AMPs, such as cathelicidins and defensins, which have fungicidal activity (Celestrino et al. 2021). Doubtlessly, human-derived AMPs such as LL-37, human β-defensin 2 (hBD-2), ribonuclease 7 (RNase 7), and psoriasin are ideally suited for developing novel therapeutics for dermatophytosis (Fritz et al. 2012; Mercer and O'Neil 2020).

Effects on *Fusarium* species

Fusarium species are hyaline saprophytic molds that are frequently reported as agents of opportunistic infections in humans. Besides this, mycotoxins produced by *Fusarium* show varying levels of toxicity to humans and animals after consumption of contaminated grain (Jacobs and Walsh 2023). In a study performed by van der Weerden et al. (2010), two agronomically important species of *Fusarium* exhibited exquisite susceptibility to LL-37. In this respect, the half-maximal inhibitory concentrations (IC_{50}) values of LL-37 against *F. graminearum* and *F. oxysporum* f. sp. *Vasinfectum* were 1 and 2.5 μM, respectively. The same authors also furnished evidence that LL-37 has fungicidal activity on *F. oxysporum* f. sp. *vasinfectum* hyphae. Kinetics studies also revealed that LL-37 induced rapid permeabilization of hyphal membrane (van der Weerden et al. 2010). This finding supports the hypothesis that LL-37 functions primarily through direct interaction with and disruption of the fungal plasma membrane. A second study involving genetically engineered Chinese cabbage found that transgenic expression of LL-37 conferred enhanced resistance to *Fusarium oxysporum* f. sp. *Lycopersici* KACC 40032 (Jung et al. 2012). Considering these encouraging results, transgenic plants expressing AMPs, like LL-37, are likely to enhance resistance against fungal pathogens and inhibit production of mycotoxins.

Effects on *Malassezia* species

Malassezia species are lipophilic yeasts that belong to the normal skin flora. One of the most commonly encountered species is *M. furfur*. It can become pathogenic under certain conditions. This phenomenon is influenced by a unique host-agent interaction that triggers the production of several virulence factors, such as various lipase enzymes, indoles, ROS, azelaic acid, hyphae formation, and biofilm formation (Kurniadi et al. 2022). *M.fufur* is associated with a variety of dermatological conditions, notably seborrheic dermatitis and tinea versicolor (Rhimi et al. 2020).

López-García et al. (2006) demonstrated that treatment of *M. furfur* with different concentrations of LL-37 led to delay or complete inhibition of fungal growth. Furthermore, NHKs that were exposed *M. furfur* for 24 h had a higher expression level of LL-37 mRNA in comparison to the untreated NHKs (López-García et al. 2006). In another work, an 18-h treatment with *M. sympodialis* triggered LL-37 secretion from monocyte-derived dendritic cells (MDDCs) obtained from atopic eczema (AE) patients (Agerberth et al. 2006). Contrarily, the exposure of MDDCs retrieved from the healthy individuals to *M. sympodialis* had no or a negative impact upon the amount of secreted LL-37. While MDDCs from AE patients showed an initial transcriptional upregulation followed by a gradual downregulation of LL-37 transcription after 1-h exposure to *M. sympodialis*, the LL-37 transcription in MDDCs from healthy individuals was uninfluenced by *M. sympodialis* (Agerberth et al. 2006). Hence, it seems reasonable to believe that *M. sympodialis* could instigate innate immune responses differently in AE patients and healthy individuals. Another conclusion is that increased secretion of LL-37 from the MDDCs in AE patients may reflect the severity of their inflammatory response to this yeast. In future studies, detailed molecular investigations will be needed to parse out the protective and therapeutic roles of LL-37 in patients with skin diseases caused by *Malasezzia* species.

Effects on *Pythium insidiosum*

The *Pythium* genus contains some notorious plant pathogens. They belong to the order oomycetes. Only *P. insidiosum* has been reported to cause disease in mammals, particularly in tropical and subtropical areas of the world. The pathogen is able to cause cutaneous, subcutaneous, intestinal and, less commonly, systemic infections. Infection is not contagious; there have been no reports of animal-animal or animal-human transmission (Daly et al. 2022). In a recent study focusing explicitly on antifungal activity of LL-37 and other AMPs (MSI-78 and magainin-2) against 14 strains of *P. insidiosum*, LL-37 exhibited growth inhibitory effects (Table 1), with minimum inhibitory concentration (MIC) values in the range of 20–40 µg/mL (Denardi et al. 2022). Future studies should examine these effects and the direct inhibition of *P. insidiosum* growth using in vivo models.

Effects on *Rhizoctonia solani*

Rhizoctonia solani is one of the most widespread soil-borne pathogens, causing massive damage to economically important crops worldwide. It is an aggressive basidiomycete necrotrophic plant pathogen with a wide host range. Symptoms associated with *R. solani* infections are diverse and change depending on the host: they include hypocotyl, crown, stem and root rot, blights, wire stem, and damping off (Dell'Olmo et al. 2023). The antifungal properties of LL-37 against *R. solani* are rarely explored. According to one study, the transgenic expression of LL-37 in genetically-modified Chinese cabbage reduced the average size of *R. solani* lesions on plant leaves (Jung et al. 2012). Since the pathogen has an almost unlimited host range, LL-37 and its derivatives are still awaiting further research in a wider variety of genetically modified plants.

Effects on *Saccharomyces cerevisiae*

S. cerevisiae is a model organism for understanding cellular processes in higher eukaryotes (Memariani and Memariani 2020). One study investigated the in vitro antifungal activity of LL-37 against three clinical strains of *S. cerevisiae*, which were isolated from vaginal infections (Scarsini et al. 2015). MIC values of LL-37 for these strains were reported to be 2 µM, suggesting that LL-37 had a potent growth-inhibitory activity. However, cellular and molecular mechanisms behind these observations remain unclear.

Antifungal effects of LL-37 on fungal biofilms

Biofilms are syntropic conglomerations of microorganisms that reside in a matrix of extracellular polymeric compounds attached to a surface. The formation of biofilms is one of the mechanisms responsible for antifungal drug resistance (Radojević et al. 2023). Similar to bacteria, biofilms bestow upon fungi a protective shelter, creating a high-level barrier against most antimicrobials. Due to the lack of effectiveness and adverse effects of existing antifungal medications, safer drugs are exigently needed. The multifunctional properties of AMPs as new therapeutics further reinforce their potential role for prevention and treatment of fungal biofilms (Oshiro et al. 2019).

Over the past decade, several studies have been conducted to investigate whether LL-37 affects different stages of fungal biofilm formation (Table 3). According to Scarsini et al.

Table 3 Antibiofilm activities of the human cathelicidin LL-37 against different fungal pathogens

Biofilm-producing strains	Identifiers	Methods	Medium for biofilm assays	LL-37 concentration ranges	Key findings	References
Candida albicans	SC5314	DNA microarray	YPD broth	5 µg/mL	• Alteration in the expression of different genes that contribute to biofilm formation (downregulated: *HYR1*, *RHR2*, and *TRY4*, upregulated: *NRG1* and *YWP1*)	Tsai et al. 2014
	SC5314	Microtiter attachment assay and fungal cell viability assay (XTT assay)	SDB	32 and 64 µM	• Significant reductions in the number of adherent viable cells by LL-37 as compared with the untreated control samples after incubation for 30 min or 48 h ($p < 0.001$) • Inability of LL-37 (at concentrations up to 32 µM) to reduce the viability of biofilm-embedded yeast cells after incubation for 48 h	Scarsini et al. 2015
	Three strains (CA 1407, CA 1408, and CA 1409, from the PAN, Wroclaw, Polish Collection of Microorganisms)	SSA biofilm formation (CV staining)	NM	100 µg/mL	• Reduction of biofilm formation in the presence and absence of DNA after 24 h, but not 48 h (LL-37 did not maintain its inhibitory effect after 48 h of incubation)	Durnaś et al. 2016
	NCTC 3179	SSA biofilm formation (CV staining) and fungal cell viability assay (XTT assay)	NM	38.6 µg/mL	• Significant inhibition of biofilm formation by LL-37 compared with the untreated control ($p < 0.0001$) • Significant inhibition of maturation of early biofilms by LL-37 as compared with the untreated control ($p < 0.001$) • Significant reduction in metabolic activity of biofilm-producing cells by LL-37 as compared with the untreated control ($p < 0.001$) • No significant effects on metabolic activity of biofilm-embedded cells	Luo et al. 2017

Table 3 (continued)

Biofilm-producing strains	Identifiers	Methods	Medium for biofilm assays	LL-37 concentration ranges	Key findings	References
	A clinical strain	Microtiter attachment assay and SSA biofilm formation (CV staining)	NM	1 to 100 μg/mL	• Reduction of fungal adhesion to microtiter wells by LL-37 (10–100 μg/mL), alone or when immobilized on MNPs • Inhibition of biofilm formation by LL-37 (1–100 μg/mL), alone or when immobilized on MNPs	Niemirowicz et al. 2017
	Dual-species biofims (*C. albicans* SC5314 + a Gram-negative bacterium): *Acinetobacter baumannii* ATCC 19606 or *Pseudomonas aeruginosa* PAO1 or *Escherichia coli* ATCC 25922 or *Klebsiella pneumoniae* ATCC 700603	Fungal cell viability assay (plate count technique)	TSB-g	100 μg/mL	• Inability to decrease the viability of microbial cells residing in 24-h old dual-species biofilms	Hacioglu et al. 2020
Candida tropicalis	A clinical strain isolated from a patient with bloodstream infection	Fungal cell viability assay (XTT assay)	RPMI 1640	0.5 to 500 μg/mL	• No significant decrease in metabolic activity of biofilm-producing cells after incubation with 0.5–40 μg/mL of LL-37 for 24 or 48 h • A dose-dependent reduction in metabolic activity of biofilm-producing cells after incubation with 100–500 μg/mL of LL-37 for 24 or 48 h	Chen et al. 2021
Candida glabrata	A clinical strain	Microtiter attachment assay and SSA biofilm formation (CV staining)	NM	1 to 100 μg/mL	• Reduction of fungal adhesion to microtiter wells by LL-37 (10–100 μg/mL), alone or when immobilized on MNPs • Inhibition of biofilm formation by LL-37 (1–100 μg/mL), alone or when immobilized on MNPs	Niemirowicz et al. 2017

CV Crystal violet; *MNPs* Magnetic nanoparticles; *NM* Not mentioned, *RPMI* Roswell Park Memorial Institute; *SDB* Sabouraud's dextrose broth; *SSA* Solid surface-associated biofilm formation (performed in 96-well microtiter plates); *TSB-g* Trypticase soy broth containing 0.2% glucose; *XTT* 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2 H-tetrazolium-5-carboxanilide; *YPD* Yeast extract peptone dextrose

(2015), short-term exposure to LL-37 (64 µM) was sufficient to reduce the number of viable *C. albicans* cells that adhered to polystyrene microtiter plates by 80%. Additionally, LL-37 exhibited anti-adhesive activity when it was added to the Sabouraud medium immediately after seeding yeasts onto uncoated silicone elastomer (SE) disks or, alternatively, when yeasts were dispensed on LL-37-coated SE disks and incubated in the peptide-free medium (Scarsini et al. 2015). The latter activity occurred in a concentration-dependent manner. LL-37 is also capable of suppressing the attachment of yeasts cells to biotic surfaces (Tsai et al. 2011a). This would result in a reduction in the number of fungal cells actually participated in the initial stages of biofilm production. In another study, LL-37 strongly suppressed adhesion of *C. albicans*, *C. glabrata*, and *C. tropicalis* to microtiter wells (Niemirowicz et al. 2017). Interestingly, the observed anti-adhesive ability of LL-37 was enhanced when it was immobilized on MNPs, suggesting a synergistic effect between MNPs and LL-37. As hinted earlier in this review, the interactions of LL-37 with both cell wall carbohydrates and proteins (Tsai et al. 2011a, b), as well as the increased β-1,3-exoglucanase activity of Xog1p, (Chang et al. 2012) are so far proposed mechanisms responsible for anti-adhesive effects of LL-37.

It has been shown that LL-37 could decrease the formation of *C. albicans* biofilm in the presence or absence of DNA after 24 h of incubation (Durnaś et al. 2016; Luo et al. 2017). However, LL-37 failed to maintain its inhibitory activity after 48 h of incubation as the biofilm mass formed at this time was comparable to that of the non-treated control (Durnaś et al. 2016). Prevention of initial microbial adhesion by LL-37 appears to be the main reason for this suppressive effect. A second reason may be changes in fungal gene expression. A DNA microarray analysis has indicated that LL-37 affects the expression levels of several biofilm-related genes (Tsai et al. 2014). Another important observation was a noticeable decline in the metabolic activity of biofilm-producing cells after treatment with LL-37 (Luo et al. 2017). In a separate study on a clinical *C. tropicalis* isolate, Chen et al. (2021) observed a dose-dependent decrement in the metabolic activity of biofilm-forming cells after incubation with high concentrations (100–500 µg/mL) for 24 or 48 h. This reduction was not observed when *C. tropicalis* was exposed to low concentrations (0.5–40 µg/mL) of LL-37.

Luo et al. (2017) highlight the effectiveness of LL-37 in inhibiting the maturation of early *C. albicans* biofilms. Nevertheless, available evidence indicates no inhibitory effects of LL-37 on metabolic activity of biofilm-encased cells (Scarsini et al. 2015; Luo et al. 2017). In another work, restriction of the growth of mature *Candida* biofilms was reported in the presence of LL-37, alone or when immobilized on MNPs (Niemirowicz et al. 2017). A recent study also demonstrated that 100 µg/mL of LL-37 was ineffective against dual-species biofilms formed by *Candida albicans* and one of the four clinically important Gram-negative bacteria (Hacioglu et al. 2020). In view of this, it will be of interest to explore if LL-37 at higher concentrations, alone or in combination with the current antifungal drugs, can decrease the viability of *C. albicans* cells enclosed in biofilms.

Antifungal mechanisms of action for LL-37

Considering the relatively large amount of experimental data on molecular mechanisms responsible for antifungal activity of LL-37, and for the reader's convenience, this section brings together and summarizes antifungal mechanisms of action of this peptide.

Two decades of research have expanded our understanding of how LL-37 exerts its growth-inhibiting and/or killing actions on a variety of fungal pathogens. In this connection, different research groups have made use of *C. albicans* as a model organism to gain molecular-levels insights into antifungal effects of LL-37. There is now sufficient experimental data to corroborate the multi-pronged nature of the attack of LL-37 on fungal cells. Fungal cell wall serves as a main target for antimicrobial action of LL-37. Interaction with cell wall components such as the major β-1,3-exoglucanase (Tsai et al. 2011a; Chang et al. 2012), reduction of both cell wall thickness and total polysaccharide content (Tsai et al. 2014), inhibition of the cell wall reconstruction, and disruption of the cell wall integrity (Luo et al. 2019) have been well described in different studies. Induction of various structural changes in fungal plasma membrane, rapid membrane permeabilization, and subsequent release of vital cellular components are the other mechanisms by which LL-37 affects fungal cell viability (López-García et al. 2005; den Hertog et al. 2006; Sonesson et al. 2007; Ordonez et al. 2014).

Aside from altering the cell wall and plasma membrane, LL-37 has a number of intracellular targets as well. In particular, expression levels of a wide array of genes were changed in response to LL-37 treatment (Tsai et al. 2014). Moreover, LL-37 was shown to affect signaling pathways related to ER stress response and cell wall integrity (Hsu et al. 2021). Inhibition of cell cycle progression, elevation of intracellular ROS levels, stimulation of oxidative stress, disruption of ER homeostasis, and formation of autophagy-like structures were also observed in LL-37-exposed fungal cells (Rather et al. 2022; Hsu et al. 2021; Menzel et al. 2017). A summary of antifungal mechanisms of LL-37 is schematically illustrated in Fig. 1.

Evidence also began to emerge that LL-37 could reduce fungal adhesion to biotic and abiotic surfaces (Tsai et al. 2011a; Chang et al. 2012; Scarsini et al. 2015), excessive inflammation (Luo et al. 2019), invasion and cytotoxicity on

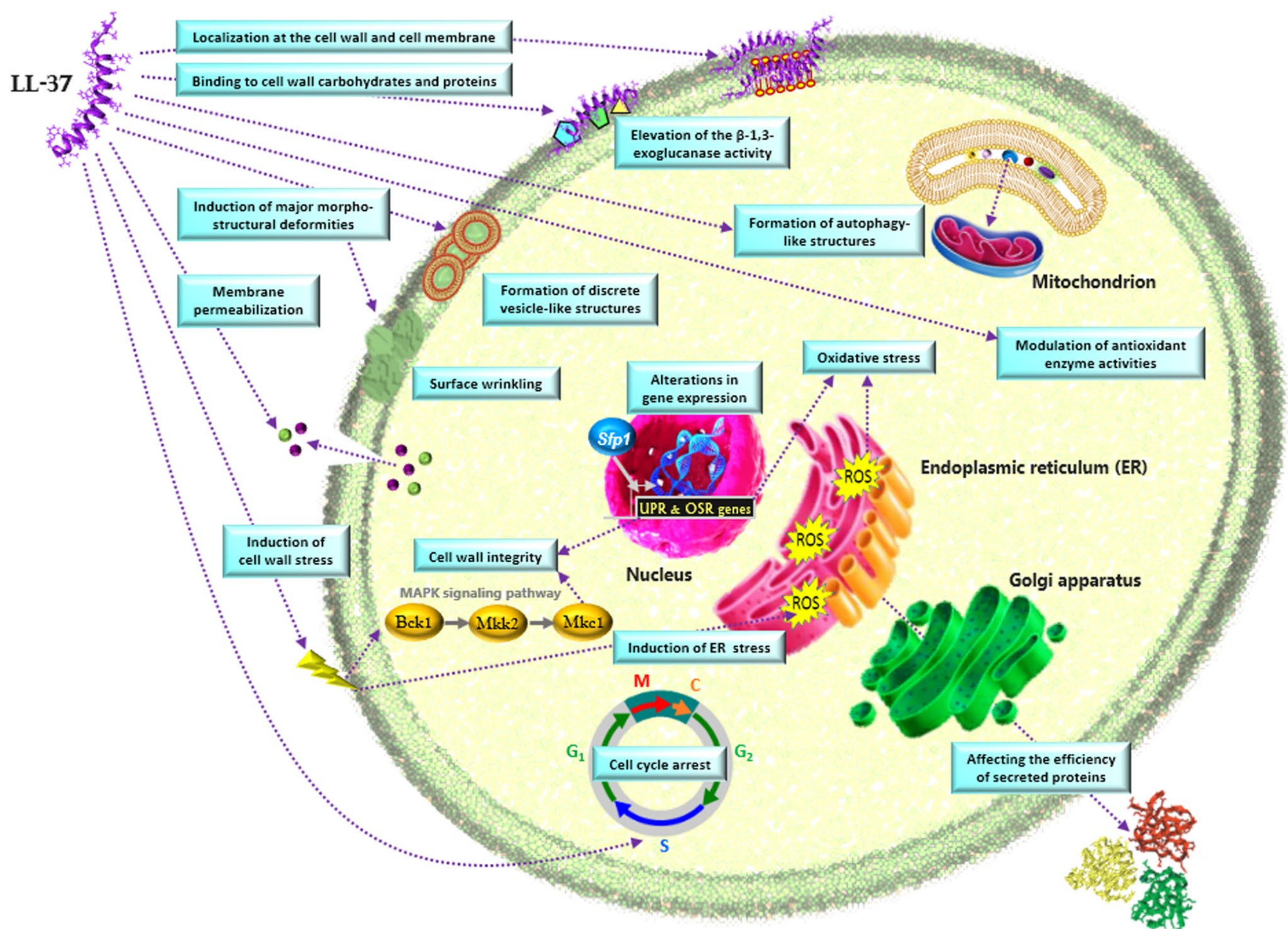


Fig. 1 Schematic representation of the molecular mechanisms underlying antifungal effects of the human cathelicidin LL-37

human cells (Luo et al. 2019), biofilm formation and maturation (Durnaś et al. 2016; Luo et al. 2017), and yeast-to-hyphal dimorphic switch (Luo et al. 2019; Wong et al. 2011). Indeed, these findings bear testimony to the notion that LL-37 may act as a potential anti-virulence peptide. In essence, quelling virulence behavior and locking fungal pathogens in a vegetative non-biofilm-producing lifestyle have been propounded as a new paradigm for the development of potential antifungal therapeutics (Reen et al. 2016; Gauwerky et al. 2009). This may make fungal pathogens less infective and more vulnerable to conventional therapy. In light of the above, it seems reasonable to embark upon in-depth investigations to assess how LL-37 interferes with fungal virulence attributes in vivo.

Potential applications and future research avenues

Due to its apparent multifunctionality, cathelicidins have been the focus of in-depth research in various fields such as microbiology, immunology, oncology, and biotechnology. The antimicrobial properties of LL-37 go beyond its role in innate immune responses and inflammation (Alford et al. 2020). Potential toxicity, susceptibility to protease degradation, and other limitations may hinder the clinical translation of LL-37, but much research is being undertaken to address them. Thus far, there have been clinical trials of LL-37 in patients with melanoma (via intra-tumoral injections) and leg ulcers (via topical route). However, no clinical trials have been conducted on antifungal effects of LL-37 (Lu et al. 2022; Dijksteel et al. 2021). In this section, we briefly discuss innovative strategies, especially various drug delivery systems, for overcoming the above-mentioned challenges and how LL-37 might be used in the future. Potential biomedical applications of LL-37 against fungal pathogens are schematically illustrated in Fig. 2.

Combination therapy with antifungal drugs or other AMPs

In view of the unique properties and activities of LL-37, it could be a promising antifungal agent when combined with

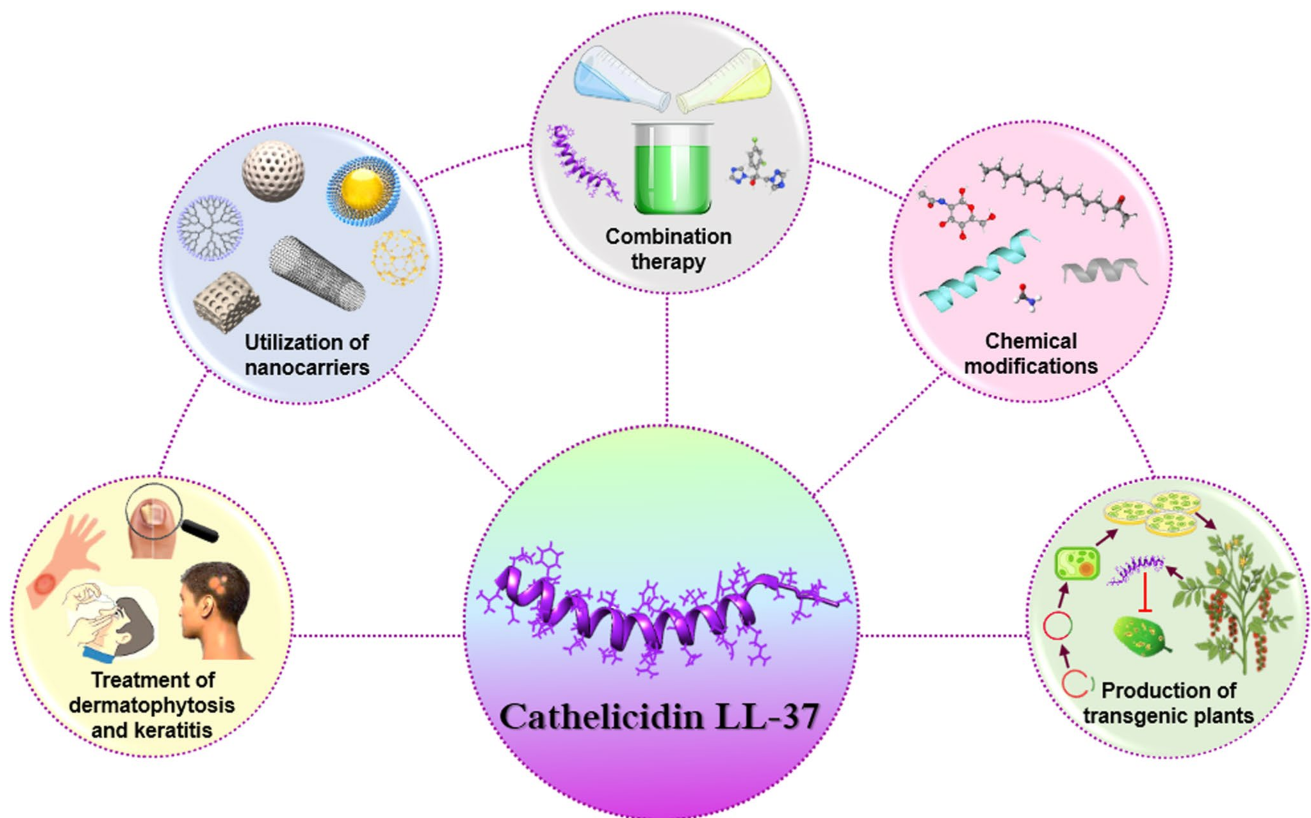


Fig. 2 Schematic representation of potential biomedical applications of the human cathelicidin LL-37 for the prevention and treatment of various fungal diseases

antifungal drugs. By combining LL-37 with existing antifungal drugs or even other AMPs, we are able to reduce the likelihood of developing drug resistance in fungal pathogens during infections. On the other hand, antifungal drugs can also cause adverse reactions including hypokalemia, infusion reaction, nephrotoxicity, hepatotoxicity, and gastrointestinal problems. Because drug toxicity is highly concentration-dependent, one way to diminish the cytotoxic effects of antifungal drugs is to combine them with AMPs like LL-37 (Fernández de Ullivarri et al. 2020). A low concentration of LL-37 could disrupt fungal cell membranes and alter their permeability, enabling other drugs with lethal effects to enter the cell. In this respect, LL-37 has recently been shown to synergize with several antifungal drugs, such as fluconazole, amphotericin B, and caspofungin in vitro (Rather et al. 2022). Nevertheless, the effectiveness of LL-37 combined with such drugs needs to be tested in animal models of fungal infections in future studies.

Chemical modifications and peptidomimetics

A potential problem associated with LL-37 is that it is easily degraded by proteolytic enzymes found in the digestive system, blood plasma, and microbial pathogens (Sieprawska-Lupa et al. 2004). Therefore, various chemical modifications of LL-37 can be made to improve its antimicrobial action and decrease its susceptibility to proteolytic degradation. Some examples of these approaches include the introduction of D-amino acids, cyclization, hybridization, lipidation, amidation or acetylation of the terminal regions (Memariani et al. 2017). In order to enhance stability of LL-37 in the serum, tryptophan or β-naphthylalanine end-tagging of the terminal regions of the peptide can also be considered (Lu et al. 2022; Gan et al. 2021; Dijksteel et al. 2021). Apart from this, some shorter analogs or fragments of LL-37 have been shown to exhibit enhanced antimicrobial activities (Kamysz et al. 2020; Yun et al. 2020). These shorter LL-37 derivatives could more readily penetrate fungal membranes and biofilm matrices.

It is also worth mentioning peptidomimetics, which have the advantage of circumventing the limitations of AMPs used in therapy. There are several advantages over AMPs, including enhanced stability, cell specificity, and better tolerability. Additionally, the synthetic flexibility of these molecules allows fast modifications of their structure in order to create novel antimicrobial peptidomimetics with particular pharmacological properties. A number of antimicrobial peptidomimetics have been developed so far, such

as β-peptides, peptoids, arylamide oligomers, β-turn mimetics, and AApeptides (Méndez-Samperio 2004). In a recent study, cathelicidin-mimetic antimicrobial peptoids based on the LL-37 structure were shown to successfully eradicate *Staphylococcus aureus* strains (Benjamin et al. 2022). Further studies with these peptoids against fungal pathogens are warranted.

Use of drug delivery systems and different formulations

As mentioned earlier, LL-37 becomes less potent when exposed to proteases secreted by host cells or microbes (Sieprawska-Lupa et al. 2004). Furthermore, LL-37 binds easily to anionic serum proteins, resulting in rapid clearance from bloodstream circulation. In order to overcome these challenges, various delivery systems such as inorganic nanomaterials (e.g., metal nanoparticles, mesoporous silica, and hydroxyapatite), polymeric materials, and liposomes have been developed to improve the efficacy of LL-37 (Lin et al. 2020).

For instance, conjugating silver or gold nanoparticles with LL-37 was shown to enhance microbicidal properties of LL-37 (Lin et al. 2020). An excellent example of polymeric materials is poly (lactic-co-glycolic) acid (PLGA). It is among the well-documented Food and Drug Administration (FDA)-approved polymers used for drug delivery systems. PLGA also displays good biocompatibility with a tailored biodegradation rate (Elmowafy et al. 2019). LL-37 encapsulated in PLGA nanoparticles was found to enhance wound healing in dermal tissue when compared to LL-37 alone (Lin et al. 2020). There are other polymeric materials that are eligible to be used for enhanced LL-37 delivery, including poly (ethyl acrylate-co-methacrylic acid) microgels, degradable anionic dendritic nanogels (DNGs), composite microgel particles based on poly-γ-glutamic acid (γ-PGA) and chitosan, sodium alginate, and collagen/hyaluronic acid polyelectrolyte multilayers (PEMs) (Cassin et al. 2016; Nordström et al. 2018; Lin et al. 2020). As for lipid-based drug delivery systems, polyethylene glycol (PEG)-coated liposomes might be served as a promising delivery system for AMPs including LL-37 (Gbian and Omri 2022). The PEG coating forms a hydration layer that inhibits the liposomes from being recognized by the reticuloendothelial system. Therefore, when LL-37 is bound to liposomes, the lipid and surrounding PEG chains provide protection against enzymatic degradation (Lin et al. 2020).

Treatment of superficial infections and keratitis

LL-37 and its derivatives are suitable for topical applications to treat superficial fungal infections of the skin, hair or nails and could be used as cosmetic ingredients to deter skin pathogens and maintain skin health. They can be used in different formulations such as ointments, creams, lotions, gels, shampoos, or wound dressings (Rodríguez-Castaño et al. 2023). Fungal keratitis has also been seen to increase due to escalating number of new contact lens wearers worldwide in recent years. A new line of research would be the study of LL-37 as eye drop formulations for the treatment of fungal keratitis (Wu et al. 2017). Certainly, these new formulations need to be evaluated in vivo.

Plant-based expression systems and transgenic plants

There is no doubt that plants are one of the most promising platforms for large-scale and cost-effective commercial production of AMPs (Mirzaee et al. 2021). Owing to many advantages over other prokaryotic and eukaryotic expression systems, plants are excellent hosts for the production of recombinant proteins. It is possible to store or lyophilize plant-based therapeutic products for a longer shelf life without the need for low temperatures to ensure stability and activity. The main advantage of plant-based expression system is the ability to perform post-translational modifications, which may be essential for protein folding and biological functions of AMPs (Shanmugaraj et al. 2021).

AMPs can be produced in plants by a variety of genetic approaches, such as whole plants, tissue specific expression, tissue culture, or transient expression. The tobacco plant (*Nicotiana tabacum*) is the most commonly used transgenic expression system (Fernández de Ullivarri et al. 2020). Transgenic plants expressing LL-37 hold opportunities for fighting fungal phytopathogens. In addition to antifungal activity, LL-37 may also be capable of protecting crops from mycotoxin contamination by inhibiting mycotoxin biosynthesis, which opens new avenues for their use in agriculture and food industry. Clearly, this direction of research appears to be worthy of further pursuance.

Conclusion

Fungal pathogens pose a formidable threat to humans, wildlife, and agriculture. The dearth of effective antimycotics in the pipeline along with the emergence of drug-resistant strains highlights the importance of antifungal drug discovery and development. LL-37, the sole human cathelicidin, has been shown to exert potent growth-inhibitory activity at micromolar concentrations against different clinically and agronomically relevant fungal strains. Furthermore, accumulating data reveal multi-pronged mechanisms of action that rely on affecting fungal cell wall and plasma membrane as well as targeting intracellular components. The promise of LL-37 as an antifungal

peptide can only be fulfilled through a thorough investigation of its in vivo efficacy and safety, and we are therefore likely to witness continued progress in this field in the coming years.

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