

Effect of MAF-1A Peptide on ALS1 And ALS3 Gene Expression in *Candida Albicans* Isolated from Vulvovaginal Candidiasis

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Abstract

Vulvovaginal candidiasis (VVC) represents a very common mucosal infection among women worldwide, and in more than 90% of cases, it is caused by *Candida albicans*. Pathogenicity in *C. albicans* is mainly related to virulence factors, among which the agglutinin-like sequence (ALS) genes play a significant role. These ALS genes, particularly ALS1 and ALS3, encode for cell surface glycoproteins that bind to host epithelial cells, necessary for biofilm formation and hyphal growth. Due to the ever-increasing emergence of antifungal resistance and failure in treatment associated with biofilms, there is a need for the exploration of new antimicrobial agents with different modes of action. Antimicrobial peptides, such as MAF-1A from *Musca domestica* (housefly), have demonstrated good antifungal activity against a broad spectrum of fungi. In this research, we investigated the effect of MAF-1A peptide on gene expression of ALS1 and ALS3 in clinical isolates of *C. albicans* obtained from VVC patients. Swabs were collected from 180 symptomatic patients and species of *Candida* were identified phenotypically and by molecular methods. The results demonstrated that 32 *Candida albicans* isolates were detected in patients with vulvovaginal candidiasis (17.8%). Susceptibility testing was carried out using MIC, and time-kill assays, while gene expression was quantified by RT-qPCR using the $\Delta\Delta CT$ method. MAF-1A showed concentration-dependent antifungal activity as complete fungicidal effect with no regrowth at 2xMIC within 2 hours. Treatment with MAF-1A significantly downregulated ALS1 expression (0.46–0.70 fold) and ALS3 expression (0.45–0.90 fold) across all three tested isolates. These results indicated a decrease in adhesion and virulence potential. It was therefore concluded that the MAF-1A peptide has dual effects against antifungal and anti-virulence, supporting its development as a promising therapeutic candidate for vulvovaginal candidiasis management.

Keywords: *Candida albicans*, vulvovaginal candidiasis, MAF-1A antimicrobial peptide, ALS genes.

Introduction

Vulvovaginal candidiasis (VVC) is a common mucosal infection that affects millions of women globally and often recurs. Although other *Candida* species are capable of causing VVC, *Candida albicans* is by far the most common etiological agent, accounting for more than 90% of VVC cases. Pathogenicity is associated with virulence factors of *C. albicans*, including the ability to switch from a yeast form into invasive hyphal forms and to form strong biofilms on mucosal surfaces of the host (Willems et al., 2020). Both processes critically depend on the adhesin genes, in particular genes of the agglutinin-like sequence (ALS) family. ALS1 and ALS3 are responsible for coding cell surface glycoproteins, without which initial adhesion to epithelial cells of the host does not take place, and subsequent hyphal formation would not occur making them primary determinants for colonization by *C. albicans* and development of disease in the vaginal environment (Ahmed et al., 2026).

The management of VVC has become increasingly complicated by the emergence of antifungal resistance and the inherent tolerance of biofilms to conventional therapies like azoles and polyenes. This has made it very necessary to have new antifungal agents with different mechanisms of action. Antimicrobial peptides have emerged as a new class of potential therapeutic agents that could help to address this challenge due to their broad-spectrum and low probability of inducing resistance. MAF-1A, an antimicrobial peptide of 26 amino acids isolated from the housefly (*Musca domestica*), has shown strong antifungal activity against *C. albicans*. Mechanistic studies indicate that MAF-1A is a multi-target fungicidal peptide that perturbs cell membrane integrity, binds intracellular nucleic acids, and affects the expression of sterol biosynthesis genes such as ERG11. Its derivatives have also been proved effective in inhibiting biofilm formation by downregulating hyphal-related genes, including ALS3 and HWP1. On the other hand,

the specific effect of native MAF-1A peptide on ALS1 and ALS3 expression in clinical VVC patient isolates needs to be directly determined (Ma et al., 2020; Cheng et al., 2026).

Thus, this study is designed to further investigate the effect of peptide MAF-1A on the expression of key virulence genes ALS1 and ALS3 in strains of *Candida albicans* isolated from patients with vulvovaginal candidiasis. In other words, the MAF-1A peptide may or may not affect how easily ALS1 and ALS3 work in different samples of *Candida* from women with vulvovaginal candidiasis.

Materials And Methods

The Peptide

MAF-1A was synthesized by Sangon Biotech (Shanghai, Shanghai, China) as a linear peptide of 26 amino acids: KKFKETADKLIESAKQQLLESLAKEMK. High-performance liquid chromatography (HPLC) confirmed purity $\geq 95\%$. The peptide was dissolved in sterile ultrapure water at 5 mg/mL and stored at -20°C .

Isolation and Identification of *Candida* species

Vaginal swab samples were taken from 180 patients who visited the three major hospitals in Baghdad, Iraq, between September 2024 and March 2025 and from whom *Candida* was isolated. The samples were collected by gynecologists. They were considered infected if they had symptoms of vulvovaginal candidiasis, i.e., vulvar and vaginal itching, edema, erythema, and clumps of vaginal discharge adhering to the vaginal wall. The vaginal swabs were inoculated on Sabouraud dextrose agar and Hi Crome™ *Candida* identification agar (Himedia, India), and then, they were incubated at 37°C for 48 hours. The identification of *Candida* species was carried out using the VITEK-2 system with reference to colony color and characteristics, microscopic morphology, and biochemical tests.

Antifungal Activity Assay

Determination of Minimal Inhibitory Concentration (MIC)

The minimal inhibitory concentration was determined using the broth microdilution method. YPD medium was inoculated with *Candida albicans* isolates (1×10^5 – 5×10^5 CFU/mL); the peptides were also diluted twofold gradient to 100-1000 $\mu\text{g/mL}$, *Candida albicans* and the diluted peptide were added at 1:1 ratio. The 96-well plate was incubated for 48 h at 28°C , after which absorbance was read at 600 nm with a microplate reader. The minimum inhibitory concentration was the lowest concentration at which there was 100% inhibition of fungal growth. The experiment was done in triplicate (Peng et al., 2022).

Time–Killing Assay

In the YPD medium, *Candida albicans* isolates (1 – 5×10^6 CFU/mL) were inoculated first and then the peptide was prepared at a dose of 100-1000 $\mu\text{g/mL}$. The peptide solution and *Candida* were mixed and cultured in a 1:1 volume. Time points of 0 min, 30 min, 1 h, 2 h, 4 h, 6 h, 12 h, and 24 h were selected. Suitable dilutions were plated on YPD agar plate for culture at 28°C for 24 h. The number of colonies was counted, and then the sterilization kinetics curve was drawn. Non-antimicrobial peptide was used as a control. The experiment was done in triplicate (Peng et al., 2022).

Molecular Study for the Isolated *Candida albicans*

Candida DNA Extraction

DNA was extracted from *Candida albicans* isolates with the Promega DNA Extraction Kit in accordance with the instructions of the manufacturer.

Candida albicans Identification

The DNA extracted was amplified with primers specific for *Candida albicans*. The primers used for the 18S rRNA gene are listed in Table 1. The amplification procedure was the same as the method described earlier (Mahmoudi et al., 2012).

Detection 18S rRNA and ALS genes by PCR

All amplification protocols for the genes under study were strictly followed as per the methods described earlier. All reagents used for amplification were purchased from Promega. The primers (Table 1) were used in quantities of 2.5 µl, and the extracted DNA was used in quantities of 5 µl (Cheng et al., 2005). Separation was performed using the 100-base DNA molecular weight standard (Promega, USA) and electrophoresis in a 1.5% agarose gel at 80 V for 1 hour .

Table 1. Sequences of the primers use to amplify ALS1, ALS3 and 18S rRNA Genes fragments.

Gene Name		Sequence 5'-3'	Product size (base per)
ALS1	F	ACCAGAAGAAACAGCAGGTG	318
ALS1	R	GACTAGTGAACCAACAAATACCAG	
ALS3	F	CCAAGTGTTCCAACAACCTGAA	184
ALS3	R	GAACCGGTTGTTGCTATGGT	
18S rRNA	F	TCTTTCTTGATTTTGTGGGTGG	150
18S rRNA	R	TCGATAGTCCCTCTAAGAAGTG	

Revelation of PCR products

The amplification products were separated by electrophoresis on a 1.5% agarose gel with 1×Tris-alkali-boric acid-EDTA buffer at 80 V for 1 hour and 30 minutes. DNA molecular weight standards (100 bp) were stained under UV light. The PCR products were developed under UV light. Thereafter, they were developed with ethidium bromide (0.5 µg/ml).

Quantitative Analysis of FKS1 Gene Expression (RT-qPCR)

The study design utilized three different *Candida albicans* isolates 7, 19, and 28 with two treatment conditions each. Hence, for each isolate, there was an untreated control group named 7, 19, and 28 and a treatment group named 237G, 237R, and 356. These treated samples were exposed to the antimicrobial peptide MAF-1A peptide. The qRT-PCR assay targeted the expression of the genes ALS1 and ALS3. For quantification of gene expression, 18S rRNA was used as the internal standard because its expression remains relatively constant under all conditions. The paper used the comparative CT method to calculate relative fold changes, so technical details like the model of the PCR instrument, reaction volumes, and denaturation, annealing, and extension temperatures and times were not provided.

Data analysis was done step by step using the $\Delta\Delta CT$ method, as explained in Table 2. In the first place, the ΔCT value for each sample was calculated. This was achieved by the difference between the CT value of the 18S rRNA reference gene and the CT value of the FKS1 target gene ($\Delta CT = CT_{ALS1} - CT_{18S}$). Subsequently, $\Delta\Delta CT$ was determined by subtracting the ΔCT of the untreated control group from the ΔCT of the corresponding treated group ($\Delta\Delta CT = \Delta CT_{treatment} - \Delta CT_{control}$). The last parameter to be calculated was the fold change in gene expression, which is equivalent to $2^{-\Delta\Delta CT}$. This gives a relative expression ratio compared to the untreated baseline.

The results obtained from Table 2 are interpreted based on the fold change values. A change value of 1.00 for all control samples indicates no change from the baseline expression level; hence, it is considered a calibration point. A value below 1.00 indicates downregulated gene expression. Therefore, it can be said that the treatment with MAF-1A peptide reduced levels of the studied gene transcript in the untreated control. This reduction can be expressed as a percentage decrease. Thus, according to the method of Livak and Schmittgen (2001), such downregulation is interpreted as a decrease in target gene expression. These results suggest that the MAF-1A peptide may exert its antifungal effect, at least in part, by suppressing studied gene expression, therefore compromising cell wall integrity in *Candida albicans*.

Results And Discussion

Isolation and identification of *Candida albicans*

The isolation and identification of *Candida* species were based on morphological characteristics (direct microscopic observation, Gram staining), culture properties, and biochemical characteristics. Swabs were inoculated onto

HiChrom Candida identification agar and SDA (continuous identification agar) for culture. Different Candida species give different colors on nutrient media; chromogenic media are therefore commonly employed for direct rapid yeast identification. This confirmed the effectiveness of HiChrom Candida agar. Strains were identified using germ tube assays, cornstarch agar morphology, and HiChrom agar. All Candida isolates showed good growth after 48 hours of culture.

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The results of isolation and identification revealed the presence of 32 Candida albicans isolates among the 180 samples from patients with vulvovaginal candidiasis (17.8%).

MIC and Time-kill assay

The minimum inhibitory concentrations for the peptide MAF-1A against 32 isolates revealed that the range of MICs was 250 to 750 µg/mL. Time-kill kinetics of MAF-1A peptide against Candida albicans isolates were evaluated at concentrations equal to the Minimum Inhibitory Concentration (MIC) and 2×MIC over 24 hours. A peptide-free culture was used as the control.

The control group (without peptide) showed logarithmic growth over the entire duration of the experiment. The viable cell count increased from 5.2 log₁₀ CFU/mL at 0 minutes to 8.3 log₁₀ CFU/mL at 24 hours. This confirmed that the experimental conditions were appropriate for the growth of the fungus.

At the MIC level, MAF-1A showed a clear but moderate fungistatic effect. The viable count decreased from an initial of 5.1 log₁₀ CFU/mL to 3.4 log₁₀ CFU/mL within the first hour and then remained almost constant at about 3.1–3.2 log₁₀ CFU/mL between 2 and 12 hours. This corresponds to around a 2 log₁₀ reduction from the initial inoculum. However, by 24 hours, as light regrowth was noticed where the count reached 3.8 log₁₀ CFU/mL, it could be said that the peptide at MIC did not totally eliminate the culture or was not able to sustain full suppression over the longer term.

MAF-1A showed strong, rapid fungicidal activity at twice the MIC (2×MIC). Viable counts dropped sharply within the first hour, from 4.7 log₁₀ CFU/mL to 1.1 log₁₀ CFU/mL. Complete killing (sterility) was achieved by the 2-hour time point, with no regrowth observed over the remainder of the 24 h; thus, the 2×MIC concentration was not only fungicidal but also effectively prevented regrowth as an indication of resistance to cells or persister survival. Complete killing (sterility, defined as 0.0 log₁₀ CFU/mL) was reached at 2 h and was sustained till the end of the 24 h monitoring period. No regrowth was observed, indicating that the 2×MIC concentration not only was fungicidal but also effectively prevented the emergence of resistant cells or persister survival.

Table 2. Time killing Assay of MAF-1A peptide against Candida albicans isolates (The results represent the average of 3 isolates; 7, 19, and 28).

Time	MIC (log ₁₀ CFU/mL)	2×MIC (log ₁₀ CFU/mL)	Control (log ₁₀ CFU/mL)
0 min	5.1	4.7	5.2
30 min	4.7	2.7	5.3
1 h	3.4	1.1	5.4
2 h	3.3	0.0	5.7
4 h	3.2	0.0	6.2
6 h	3.1	0.0	6.7

12 h	3.2	0.0	7.5
24 h	3.8	0.0	8.3

Results of the time-kill assay further describe the pharmacodynamic profile of the MAF-1A peptide against *C. albicans*. It is an antimicrobial effect that is clearly demonstrated to be concentration-dependent. The MIC concentration was able to suppress growth effectively compared to the control (where viability was reduced by ~2 log₁₀) but did not completely eradicate the organism, allowing slight regrowth at 24 hours. This indicates that at MIC, MAF-1A is mainly acting as a fungistatic agent that is, it slows growth but does not induce a full lethal hit on the whole population (Cheng et al., 2021).

On the other hand, at a concentration of 2×MIC, a rapid and sustained fungicidal effect was observed, attaining >3 log₁₀ reduction (from 4.7 to 1.1) within 1 hour and complete killing (sterility) by 2 hours. Such rapid action is typical for many antimicrobial peptides (AMPs) that kill by breaking the integrity of the fungal cell membrane and hence lead to a rapid death of the cell (Shaban et al., 2023).

No regrowth at 2×MIC is very important. It means that a concentration just two times the MIC not only kills the initial inoculum but also does not allow outgrowth of resistant mutants, a very important factor in treatment for invasive candidiasis. Complete kill at 2×MIC indicates that MAF-1A has a quite steep dose-response curve. Many common antifungals (for example, the azoles) are slowly fungistatic and need long exposure (Binder et al., 2019). Rapid kill by MAF-1A at 2×MIC (in 2 hours) is an added advantage as it may bring about faster clinical resolution and reduced risk of resistance development. However, further studies on it for safety, stability in physiological fluids, and efficacy in *in vivo* models are warranted.

In summary, MAF-1A has rapid and concentration-dependent anti-*Candida* activity. The MIC concentration is primarily suppressive, while the 2×MIC concentration is robustly fungicidal and prevents regrowth. These findings further support the continued development of MAF-1A as a promising candidate for antifungal therapy. It is recommended that therapeutic dosing should aim to achieve at least 2×MIC levels to ensure fungicidal outcomes and prevent relapse.

Detection of *Candida albicans* using the 18S rRNA gene

The 16 *Candida albicans* isolates used in this study, especially those with strong biofilm-forming ability and resistance to fluconazole, were all detected by PCR using the 18S rRNA gene. All clinical *Candida albicans* isolates tested contained the 18S rRNA gene (150 bp).

Detection of ALS genes

The PCR amplification and agarose gel electrophoresis confirmed the presence of the ALS1 and ALS3 virulence genes in the *Candida albicans* isolates tested.

PCR amplification using primers specific to the ALS1 gene yielded a single, clear amplicon of approximately 318 base pairs in lanes 2 through 5. This band corresponds to the expected product size for the ALS1 gene and matches the positive control in lane 1. The band's presence in these lanes confirms that the ALS1 gene is present in the tested *C. albicans* isolates. Lane M is where the 100 bp DNA ladder, used for size determination, was loaded; there was no non-specific amplification or primer-dimer formation.

Similarly, the results for the ALS3 gene are demonstrated as well in which a specific amplicon of approximately 184 bp was successfully generated from the *C. albicans* isolates in lanes 1 to 10. No amplification in the negative control (Lane NC) gives further evidence of the absence of contamination as well as the specificity of the PCR assay.

Amplicons of the expected size for both target genes confirmed that *C. albicans* isolates used in this study carry the ALS1 and ALS3 genes. These genes, identified as major components within their virulence repertoire, were successfully amplified.

Figure 1. The PCR amplification and Agarose gel electrophoresis results for detecting *C. albicans* virulence gene (ALS1). Lanes 1-5: *C. albicans* (318 bp); Lane 1: 100 bp Ladder. (1.5% agarose gel at 8 volt /cm for 60 minutes).

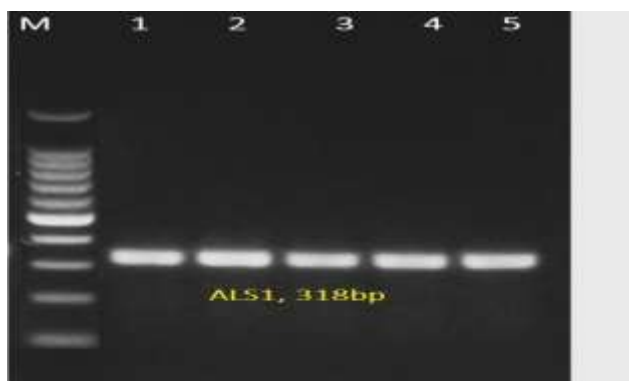
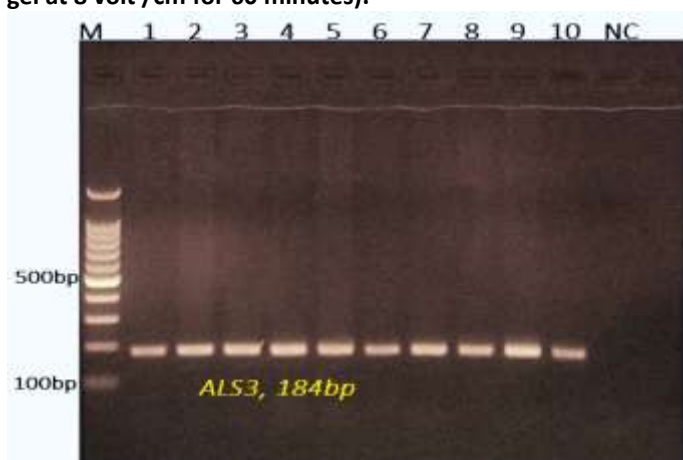


Figure 2. The PCR amplification and Agarose gel electrophoresis results for detecting *C. albicans* virulence gene (ALS1). (318 bp); Lanes 1-10: *C. albicans* (318 bp); Lane 1: 100 bp Ladder. Lane 11: Negative control. (1.5% agarose gel at 8 volt /cm for 60 minutes).



The PCR detection of the ALS1 and ALS3 genes in the *C. albicans* isolates would be a very important result if obtained, as it would support the current knowledge of these genes as major virulence factors. The ALS (Agglutinin-Like Sequence) gene family encodes large glycoproteins on the cell surface, which are essential for the pathogenicity of *C. albicans* mainly by adhesion to host tissues and surfaces. This primary adhesion is the first step in colonization, biofilm formation, and establishment of infection (Ardehali et al., 2019).

Previous research has underscored the clinical relevance of ALS1 and ALS3 expression. In a study of recurrent vulvovaginal candidiasis (RVVC), it was found that isolates from RVVC patients exhibited significantly higher expression of both ALS1 and ALS3, as compared to asymptomatic colonizers; this strongly correlated with enhanced biofilm formation. The association of these genes with antifungal resistance is becoming clearer (Yan et al., 2025). For itraconazole-resistant *C. albicans* in onychomycosis patients, another 2025 study found that there was a statistically significant upregulation of the ALS3 gene in resistant isolates; ALS1 showed no significant difference. Some other studies have associated the presence or expression of ALS1 with fluconazole resistance; those studies have suggested that the role of ALS1 in resistance may be context-dependent or strain-dependent. A robust biofilm, in part mediated by ALS genes, is known to function as a physical barrier that reduces drug penetration and contributes to therapeutic failure (Nouraei et al., 2025).

The presence of these genes confirms the genetic potential of the isolates for adhesion, biofilm formation, and a possible antifungal resistance, which is in line with their well-described functions as key virulence factors. To support this finding, future studies should use quantitative PCR (qPCR) to quantify gene expression levels, which will provide a more dynamic picture of the virulence potential of the isolates (Marek et al., 2021).

The $\Delta\Delta C_t$ method was used to determine the levels of expression of ALS1 and ALS3 genes in three *Candida albicans* isolates (C7, C19, and C28). The untreated CA isolate was set as the calibrator (fold change = 1). An endogenous control of 18S rRNA gene was used.

Treatment with MAF-1A reduced the expression of ALS1 in all three isolates, as presented in Table 1: 0.46 (CA7), 0.70 (CA19), and 0.66 (CA28). This indicates a decrease in gene expression ranging from 30% to 54% compared to the control.

Similarly, ALS3 expression was downregulated in all treated isolates (Table 2) with fold changes of 0.45, 0.90, and 0.76 (C7, C19, and C28). The most pronounced reduction was observed in CA7; a modest 10% decrease was seen in CA19, and in CA28, it was 24%.

Table 3. The level of gene expression of ALS1 gene before and after treating with MAF-1A peptide for three *C. albicans* isolates by delta delta CT method.

The sample	Mean Ct ALS1	Mean Ct 18S rRNA	Δ Ct	$\Delta\Delta$ Ct	Fold change
CA (Control)	25.14	20.08	5.05	-0.34	1
CA7	27.23	21.06	6.17	1.04	0.46
CA19	25.55	20.00	5.55	0.15	0.70
CA28	26.12	24.09	2.03	-2.38	0.66

Table 4. The level of gene expression of ALS3 gene before and after treating with MAF-1A peptide for three *C. albicans* isolates by delta delta CT method.

The sample	Mean Ct ALS3	Mean Ct 18S rRNA	Δ Ct	$\Delta\Delta$ Ct	Fold change
CA (Control)	25.14	20.08	5.05	-0.34	1
CA7	27.23	21.06	6.17	1.04	0.45
CA19	26.10	20.89	5.21	-0.18	0.90
CA28	25.53	20.09	5.44	0.04	0.76

In this study, treatment with MAF-1A peptide reduced the expression of two major adhesion genes, ALS1 and ALS3, in *C. albicans* isolates. The proteins encoded by both genes are agglutinin-like sequence (Als) proteins; these play a critical role in adhesion to host surfaces and biofilm formation, as well as virulence. The observed reductions in ALS1 (0.46-0.70-fold) and ALS3 (0.45-0.90-fold) expressions suggest that MAF-1A may interfere with the capacity for adhesion of *C. albicans*. This is akin to previous reports on how antimicrobial peptides (AMPs) can suppress the expression of virulence factors without directly killing the fungus (Luo et al., 2020; Rai et al., 2022). The effect was more consistent for ALS1 across all isolates than for ALS3, which was highly variable, especially in CA19. This may be an isolate-specific response, reflecting genetic or epigenetic differences in the MAPK signaling pathways that regulate ALS gene expression (Romani et al., 2020).

The downregulation of ALS3 is particularly relevant since Als3 is also a key invasion adhesin, which mediates endocytosis into host cells. Thus, MAF-1A may reduce both adhesion and invasion, supporting its potential as an antivirulence agent. Results from previous studies with other peptides, such as LL-37 and histatin-5, have also shown suppression of ALS3 expression in *C. albicans*. However, MAF-1A appears to work at lower concentrations and across multiple isolates, pointing to a broader therapeutic window.

The study is limited by the relatively small number of isolates. Further work should encompass a larger panel of clinical isolates and phenotypic assays to confirm the functional consequences of the observed transcriptional changes. Phenotypic assays should include biofilm formation and adhesion to epithelial cells. An investigation of the molecular mechanism through which MAF-1A acts, such as whether it binds directly to transcription factors like Bcr1 or Tec1, would also be of interest (Nobile & Johnson, 2015; Fox et al., 2020). The MAF-1A peptide significantly reduces ALS1 and ALS3 expression in *C. albicans*, thus supporting its further development as an anti-adhesive and anti-biofilm agent for use against this organism. Many previous local studies indicated to the role of antimicrobial

peptides as growth inhibitors for the microorganisms and down regulated for the virulence genes (Kareem and Ghaima, 2024; Khalaf and Ghaima, 2026).

Conclusion

MAF-1A peptide exerts potent concentration-dependent antifungal activity against clinical isolates of *Candida albicans*, with rapid fungicidal effects at 2×MIC and sustained suppression without regrowth. It significantly downregulates the expression of two key ALS genes, ALS1 and ALS3, which play a crucial role in adhesion, biofilm formation, and invasion of host tissue. Thus, these results confirm the dual mechanism of MAF-1A as a fungicidal agent and an anti-virulence compound. More comprehensive studies involving larger panels of isolates and in vivo models are needed to validate its potential for the treatment of vulvovaginal candidiasis.

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