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Effect of *Candida albicans* Co-culture on *Metalloprotease 1* Gene Expression in *Microsporum canis*

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ABSTRACT

Fungi that share a common clinical habitat may interact with or antagonize each other. Chemical molecules produced by *Candida albicans* have inhibitory effects on the mycelial growth of dermatophytes. The present study investigated the effect of *C. albicans* on the expression of metalloprotease (MEP) genes in *Microsporum canis* isolated from clinical samples. This study aimed to investigate the effect of *C. albicans* on *M. canis*. *C. albicans* was initially identified using molecular methods and phenotypic tests. Then, it was co-cultured under laboratory conditions in the presence of *M. canis*, which is capable of expressing pathogenic genes. Subsequently, the effect of *C. albicans* growth on the expression of dermatophyte genes was investigated using real-time PCR (RT-PCR). In conclusion, co-culture with *C. albicans* showed an approximately 62% reduction in *metalloprotease 1 (MEP1)* gene expression in *M. canis* in the examined sample. Therefore, it seems that the inhibition of hyphomycete growth is mediated by fungal compounds themselves, which represents a controversial area for future research.

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Abbreviations

C. albicans, Candida albicans; Ct, Cycle threshold; MEP1, Metalloprotease 1; PCR, Polymerase chain reaction; PSZ, Posaconazole; QS, Quorum sensing; RT, Reverse transcription; SDA, Sabouraud Dextrose Agar; TER, Terbinafine; VOR, Voriconazole

Introduction

Candida albicans (*C. albicans*) is an opportunistic fungal pathogen that causes a broad spectrum of infections in animals [1]. Recent studies have reported microbial chemocommunication through chemical signaling molecules, such as (2E,6E)-farnesol, which is secreted by *C. albicans* [2]. Sesquiterpene alcohols produced by *C. albicans* have been shown to inhibit hyphal formation and may contribute to the suppression of dermatophytosis [2-4]. Due to the limitations associated with synthetic antifungal agents, including high production costs, adverse effects, and the emergence of drug resistance, current research is increasingly focused on natural antifungal compounds [5]. Chemical compounds such as sesquiterpenes found in certain plant essential oils have emerged as promising candidates [6]. Their derivatives exhibit multiple biological activities, including antibiofilm, anticancer, antitumor, and fungicidal properties [7,8], and also demonstrate antibacterial activity against various veterinary bacterial isolates *in vitro* [7, 9-10]. Moreover, sesquiterpene alcohols appear to possess anti-inflammatory properties, which may be beneficial in the treatment of otitis externa and other inflammatory conditions [11-13].

Although fungal species may compete for the same substrate, such as skin, it remains unclear whether they can directly inhibit each other's growth [14]. *C. albicans* is one of the most frequent causes of superficial and systemic fungal infections in susceptible animals. Quorum sensing (QS), mediated by both internal and external molecules, is recognized as an important regulatory mechanism in microbial behavior, particularly among fungi. These molecules influence virulence, growth, and antifungal resistance [15-17]. Farnesol, an external QS molecule secreted by C. albicans, has demonstrated inhibitory effects on dermatophyte growth [2] and plays a role in modulating fungal virulence traits [3].*Microsporum canis* (*M. canis*), a keratinophilic hyphomycete, is a well-documented causative agent of ringworm infections in animals and is responsible for a large proportion of dermatophytosis cases in dogs [18].

In the present study, a strain of *C. albicans* was co-cultured with *M. canis* under in vitro conditions to assess its influence on the expression of dermatophyte pathogenicity-related genes.

Materials and Methods

Sample Collection

Clinical samples suspected of containing *C. albicans* were collected from dogs at a veterinary clinic in Tehran using sterile, capped containers containing cryopreservative medium (QLabe, Canada). The samples were stored at 4 °C and transported to the microbiology laboratory for further analysis.

Phenotypic Tests for C. albicans

All yeast isolates were initially cultured on Sabouraud agar (Sigma-Aldrich). The cultured isolates were then inoculated onto Corn Meal Agar (QLabe, Canada) containing 1% Tween 80 and into 0.5 mL of human serum. The presence of chlamydoconidia and germ tubes was examined using a microscope at 40× magnification.

Molecular Identification of C. albicans

DNA was extracted using the CinnaPure-DNA Extraction Kit (SinaClon, Cat. No. PR881612; 50 preparations). A target DNA fragment was amplified by polymerase chain reaction (PCR) using the specific primers listed in Table 1. The reaction mixture contained template DNA, primers, the four deoxynucleotide triphosphates, a thermostable DNA polymerase, and an appropriate reaction buffer. Amplification was performed under optimized thermal cycling conditions [18].

Table 1. Primer specifications of ALs in C. albicans.

Gene	Nucleoid Sequence	bp
ALS	F-5'-ATGAAGTCTACCATCTTCTCCG-3'	122
	R-5'-TCCGCTTCTGTCGGTCA-3'	

Co-Culture of C.albicans and M. canis

Candida albicans and Microsporum canis isolates were cultured *in vitro* on Sabouraud Dextrose Agar (SDA) supplemented with chloramphenicol (QLabe, Canada). After 24 hours of incubation, colony morphology was evaluated macroscopically and microscopically. A reference strain of *M. canis* (ACTC 14283) was obtained from the Iranian Research Organization for Science and Technology. The *M. canis* cultures were divided into two groups: a control group containing *M. canis* alone and a treatment group containing *M. canis* co-cultured with C. albicans [19].

Determination of Metalloprotease Expression Level of *M. canis* by RT- PCR from Two Control and Treatment Groups

RNA Extraction

Total RNA was extracted using the RNeasy Micro Kit (Qiagen) and used for subsequent amplification reactions. Yeast suspensions in the logarithmic growth phase (OD600 = 0.4-0.6) were used for RNA extraction. Genomic DNA was removed using a DNase kit (Qiagen).

cDNA Synthesis

cDNA synthesis was performed using AMV reverse transcriptase at a concentration of 25 U/µL (Roche). The extracted RNA was first incubated at 65 °C for 3 minutes. Reverse transcription was then performed at 42 °C for 60 minutes in a reaction mixture containing 2 µL of random primers, 0.8 µL of AMV reverse transcriptase, 2 µL of 10 mM dNTPs, 1 µL of RNase inhibitor, and 2 µL of 10× AMV reaction buffer. Finally, the AMV reverse transcriptase was inactivated by incubation at 99 °C for 5 minutes.

Analysis of RT-PCR Products

The expression ratio of the gene of interest to the reference gene was calculated using a formula based on amplification efficiency and differences in cycle threshold (Ct) values. The Q-Gene program, an Excel-based comparative tool, was used for data input, processing, statistical analysis, graphical presentation, and normalization of gene expression data.

Results

Phenotypic and Molecular

Candida albicans was confirmed by the presence of germ tubes and chlamydoconidia. Following PCR, gel electrophoresis of the PCR products revealed bands of 122 bp.

RT-PCR Analysis

RT-PCR analysis showed that the optimal melting temperature for the *Metalloprotease 1* (*MEP1*) amplicon of *M. canis* was 86.79 °C (Figure 1). Amplification curves positioned further to the left corresponded to lower Ct values and, consequently, higher gene expression levels. Based on the curves presented in Figure 2, the expression levels of the target genes, from highest to lowest, were S-Non, S-Treat, Als-Non, and Als-Treat.

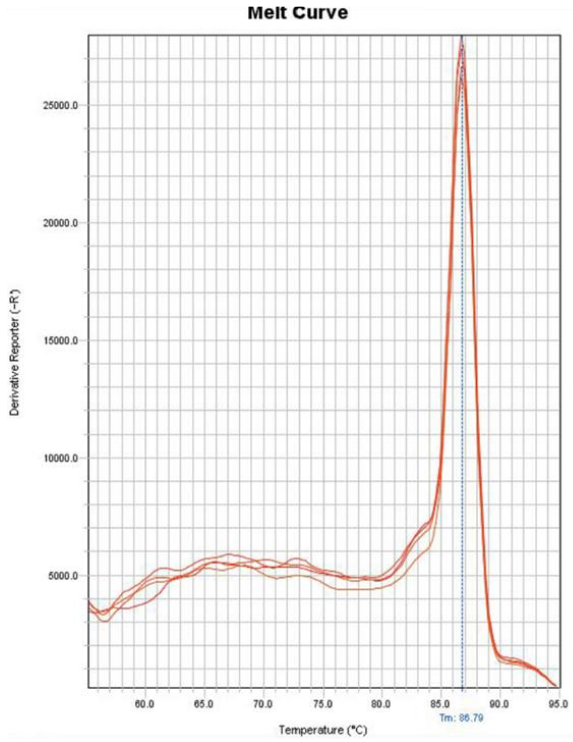

Figure 1.The results of melting curve temperature for MEP1gene of M. canis.

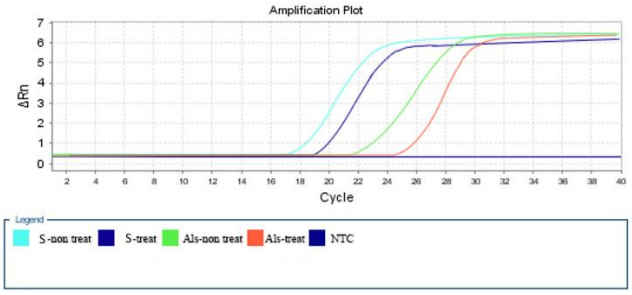

Figure 2. RT-PCR results showed that MEP1 gene expression M. canis and ALs gene expression C. albicans.

RT-PCR analysis further showed that *MEP1* gene expression was lower in *M. canis* co-cultured with *C. albicans* (S-Treat) than in *M. canis* cultured alone (S-Non). Similarly, the expression of the ALS gene in *C. albicans*, which is involved in hyphal formation, was reduced under co-culture conditions (Als-Treat).

These findings indicate that co-culture may reduce metalloprotease gene expression in the examined isolates. Overall, co-culture with *C. albicans* resulted in an approximately 62% reduction in *MEP1* gene expression in *M. canis* (Figure 3).

CT.txt

```
Ct- S -Non= 18.01
Ct- S -treat= 19.2
Ct-Als-non= 22.71
Ct-Als-treat=25.3
```

ΔCt Value (Experimental)	ΔCt Value (Control)	Delta Delta Ct Value	Expression Fold Change
ΔCTE	ΔCTC	ΔΔCt	2^-ΔΔCt
6.10	4.70	1.40	0.378929142
		% Knockdown	62.10708584

Figure 3. Results fold change in MEP1 gene expression M. canis.

Discussion

Yeast-like fungi of the genus Candida are commonly found in the mucosal microflora of animals and birds, where they can persistently colonize the skin and mucous membranes. Recent studies indicate an increasing incidence of candidiasis in both human and veterinary populations [20-23]. Candida species are also known to participate in microbial communication through quorum-sensing mechanisms [24,25]. Dermatophytes of the family Arthrodermataceae, belonging to the order Onygenales, have co-evolved with mammalian hosts and are common causative agents of cutaneous fungal infections [26].

In this study, we evaluated the expression of metalloprotease genes in *M. canis*, which are considered key virulence factors. Co-culture with *C. albicans* resulted in an approximately 62% reduction in metalloprotease gene expression in *M. canis* in the examined sample. Brouta *et al.* (2002) described the fungalysin gene family in *M. canis*, which includes *MEP1-MEP3*, with *MEP3* exhibiting collagenolytic, elastinolytic, and keratinolytic activities [27,28].

Subsequent studies identified *MEP4* and *MEP5*, expanding the family to five members (*MEP1-MEP5*), which are also conserved in Trichophyton rubrum and T. mentagrophytes [29-31].

Using enantioselective gas chromatography combined with mass spectrometry, Brasch *et al.* (2014) identified eight farnesol-related compounds. Among these, (3R,6E)-2,3-dihydrofarnesol (R-DHF), (3S,6E)-2,3-dihydrofarnesol (S-DHF), and (2E,6E)-farnesol (F-ol) exhibited potent inhibitory effects against various dermatophytes during a 62-hour incubation period [2,3,32]. These compounds demonstrated species-specific activity against *T. rubrum*, *T. mentagrophytes*, *M. canis*, and Epidermophyton floccosum [2,32], with S-DHF and F-ol showing antifungal efficacy comparable to or greater than that of fluconazole.

In studies of superficial fungal conditions, such as canine otitis externa caused by *Malassezia pachydermatis* biofilms, farnesol demonstrated strong antibiofilm activity at concentrations above 1.6 µM/mL, with optimal activity observed at concentrations ranging from 12.5 to 200 µM/mL [3]. Fungal enzymes, particularly catalase and lipase, appear to be associated with lesion development, although their expression patterns may vary depending on the fungal species and infection status [30]. Therefore, continuous surveillance of fungal species and their virulence profiles is essential for guiding antifungal therapy. Drug selection should take into account species-specific susceptibility, pharmacokinetic properties, and host-related clinical factors [3]. Voriconazole (VOR), terbinafine (TER), and posaconazole (PSZ) remain among the most effective agents for treating dermatophytosis in both humans and animals [33].

Conclusion

The presence of *C. albicans* colonies reduced the expression of *M. canis* virulence genes by approximately 62% under in vitro conditions. These findings suggest that sesquiterpene alcohols produced by *C. albicans* may reduce the pathogenicity and biofilm-forming capacity of *M. canis*, highlighting their potential value in the development of future antifungal treatments. The results also provide further insight into the possible role of quorum sensing in antifungal interactions.

Declarations

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No artificial intelligence services were used to prepare this manuscript.

Authors' Contributions
AK Conceived the study, performed data analysis, and drafted the manuscript. BK Provided clinical insights and reviewed the manuscript. AK, BK and FK Contributed to study design and data interpretation.

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Conflicts of Interest
The authors declare that they have no competing interests.

Ethics Approval
Not applicable.

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