

# Bacterial activity on hyphal formation of *Candida albicans*

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## Abstract

*Candida albicans* is an opportunistic pathogen that is commonly found in the human microflora. Virulence activity of pathogens is characterized by biofilm formation, which is characterized by *Candida albicans*. This study aimed to find the effect of interaction between *Candida albicans* and some bacteria and their effects on virulent factors of *C. albicans* such as biofilm formation and hyphal development genes (Sap5 and Ece1). The co-cultivation was performed by mixing each of the bacteria species: *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Pseudomonas aeruginosa* with *C. albicans* for biofilm conditions. The biofilm formation of *Candida* isolates was detected by the crystal violet assay in a microtitre-plate reader at 620 nm. In this study, Sap5 and Ece1 mRMA expression were indicators for hyphal formation of *C. albicans* under bacterial action. The optical density (OD620) of *C. albicans* alone was 0.08, while the OD620 of the biofilm quantification in co-cultured *C. albicans* mixed with the different bacteria species: *P. aeruginosa*, *E. coli*, *S. aureus*, and *S. pyogenes* was 0.06, 0.07, 0.09, and 0.27, respectively. The expression level of the Ece1 gene when *C. albicans* mixed with *E. coli* and *S. pyogenes* showed more decreased Ece1 mRNA expression, while the mRNA level of the Sap5 gene was significantly reduced by *C. albicans* mixed with *P. aeruginosa*. During the early stages of biofilm development, *C. albicans* co-cultured with bacteria showed significant differences in biofilm formation and the transcription levels of hyphae-specific genes.

**Keywords:** *Candida albicans*; bacteria, biofilm formation; gene expression.

## INTRODUCTION

*Candida* species is a part of the human microflora and becomes pathogenic when certain conditions exist and result in an opportunistic infection [1]. There are two major forms of *C. albicans* infections in humans: superficial infections and systemic infections, which can be fatal.

A biofilm is a consortium of microbes attached to a biotic or abiotic substrate embedded within a matrix of extracellular polymeric substances. For many microbes, the ability to form biofilms is an important virulence factor. The biological significance of the biofilm in *C. albicans* is responsible for protecting the bacteria by delaying its physical destruction and acting as a protective barrier against host immune substances and the penetration of anti-fungal agents [2].

The formation of biofilm in *C. albicans* occurs in three stages. The first stage is adherence to a suitable temperament basal layer; this is initiated after the start of incubation. This initial adherence stage is regulated by non-specific factors, such as hydrophobic or electrostatic interaction, and specific factors through specific stages is the process in which the adhered *C. albicans* continuously multiply and the yeast cells transform into hyphae that then form three-dimensional structures. The three-dimensional structures of biofilm are generally comprised of yeast, pseudohyphae, and hyphae [3].

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The last maturation stage is the process of quantitative increase of extracellular substances; the mature biofilm enables the *Candida* yeast to fix biofilm onto the extracellular surface, and the hyphae form a cross-sectional structure with structural frames. The dimorphism of *C. albicans* serves as a major factor that influences the formation of biofilm [4].

Depending on the localization of the microbial communities [5], *C. albicans* colonizes various habitats of the human body, representing various conditions in terms of interactions with bacteria species. Synergistically, the metabolic interactivity between *C. albicans* and bacteria adopts specific adhesion activity for yeast hyphal development [6]. This study showed the importance of expression levels of hyphal development genes *Sap5* and *Ece1* in the co-culture of *C. albicans* and bacteria conditions.

## Materials and Methods

### Sampling

A total of 100 samples were divided on the basis of infection locations: 20 oral swabs, 20 vaginal swabs, 40 urine, and 20 stools for isolated *C. albicans* and bacterial species. Samples were collected from patients suffering from vaginal candidiasis, oral thrush, and urinary tract infections who attended the teaching hospital for pediatrics in Samawa City, with an age range of 1 to 50 years. This study was approved by the ethics committee of Al-Muthanna University.

### Isolation and identification of yeast and bacteria

Using a light microscope, the yeast cells under the microscope appear as cocci with a large size, and after adding serum for the growth of *C. albicans*, they produce germ tubes. To isolate and identify *C. albicans* on sabouraud dextrose agar and CHROM agar were used, and the colony of *C. albicans* appeared green.

The biochemical test for the detection of *C. albicans* was used by API candida to explain the ability of candida to utilize different types of sugar and assure the identification of the morphological, cultural, and biochemical characteristics for differentiation among *Candida* species.

### RNA isolation and RT-PCR

Total RNA was extracted from the *C. albicans* culture cells and co-cultured with bacteria by using the Presto™ Mini gDNA Yeast Kit according to the manufacturer's instructions. To ensure the degree of existence, RNA templates were used in agarose gel electrophoresis and RNA concentrations were measured using a Nanodrop-1000 UV-VIS spectrophotometer (Thermo Fisher Scientific Inc., USA). The reverse transcription PCR cDNA synthesis was carried out using EasyScript One-step Gdna Removal and cDNA Synthesis SuperMix Kit. The RT-PCR conditions were one cycle of 42°C for 30 min, and 85°C for 5 sec.

### Biofilm quantification

Suspensions of bacteria and *C. albicans* were added to wells in a 96-well microtiter plate. The plate was incubated for 1.5 h at 37°C. After the initial adhesion phase, the cell suspensions were aspirated, and each well was washed twice with PBS to remove loosely adherent cells. The amount of biofilm formed was measured using the ELISA assay with absorbance at 620 nm [7].

### Real-time PCR assay

Real-time PCR detection of mRNA transcripts was performed by using Mx3000p (Agilent Technologies, USA). The PCR components were prepared by 10 Mm primers used to amplify the *Sap5* gene in the forwarding direction 5'-CCAGCATCTTCCCGCACTT-3 and reverse direction 5'-GCGTAAGAACCGTCACCATATTTAA-3, and *Ece1* gene in forwarding direction 5'-CCAGAAATTGTTGCTCGTGTTG-3' and reverse direction 5'-CAGGACGCCATCAAAAACG-3'. This experiment involves amplification of the Beta-Actin gene as a housekeeping gene in the forwarding direction 5'-CGTCGGTAGACCAAGACACC-3' and reverse direction 5'-CCCAGTTGGAGACAATACCGT-3', 10 µl of Luna universal qPCR master mix and 200 ng of cDNA were used as a template, and nuclease-free water was added up to a final volume of 20 µl. The PCR conditions were 94°C for 30 sec, 45 cycles of 94°C for 5 sec, 60°C for 30 sec. Subsequently, a dissociation curve was applied with one cycle at 95°C for 1.0 min, 55°C for 30 sec, and 95°C for 30 sec.

### Statistical analysis

Statistical analysis was carried out using SPSS version 24, where the rates were tested using the Chi-square test at a significant level 0.05, and descriptive statistics were calculated to find the standard deviation at a level of significance of  $p < 0.05$ .

## Results and discussion

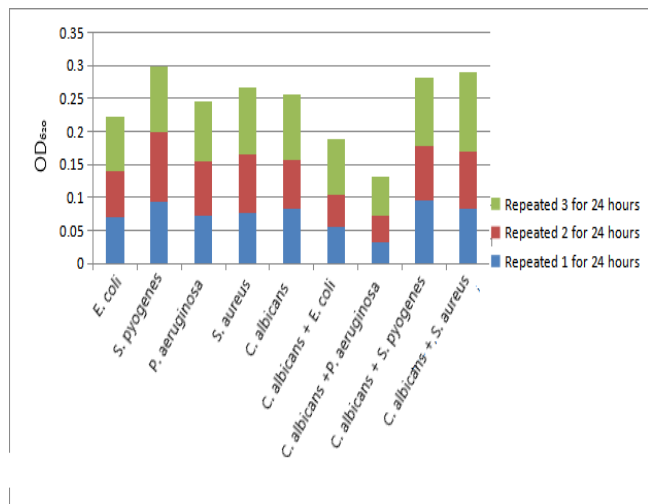
### Identification of yeast and bacteria isolates

The results revealed that the percent of *C. albicans* isolates were (19%, 18% and 9%) from oral thrush, vaginal swabs and urine, respectively. The results of the statistical analysis test showed significant differences ( $P \leq 0.0001$ ) between the percentage of samples. Darogha reported that *C. albicans* has the highest infection rate, with 31.1% of vaginitis (8). *C. albicans* was found to be the causative fungus in 50-70% of all candiduria isolates in studies (9,10).

### Biofilm Formation

The biofilm for *C. albicans* incubated alone and when it was incubated along with four types of bacteria were compared. When *C. albicans* was co-cultured with gram negative bacteria for 24 hours, its biofilm formation ability was lower than that of gram positive bacteria. The optical density (OD) were  $OD_{620} = 0.08$  and 0.06 of *C. albicans* culture alone and

co-cultured *C. albicans* with *Pseudomonas aeruginosa* for 24 hours, respectively (Figure 1).

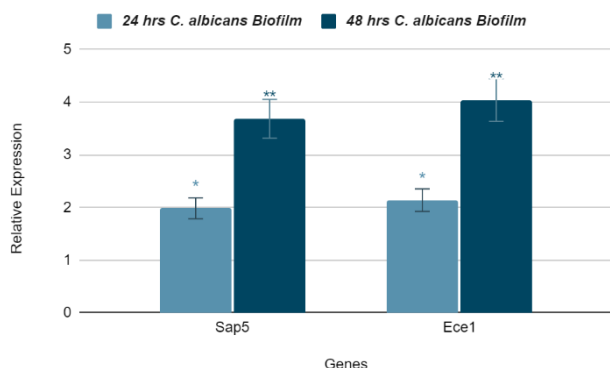


**Figure (1):** Biofilm formation of *C. albicans* in co-culture with *E.coli*, *S.pyogenes*, *P.aeruginosa*, *S.aureus*. Repeated for 24 hours.

In this study, the reduction of *C. albicans* biofilm formation was more associated with co-culture with *P. aeruginosa*, which was consistent with the previous study that stated the ability of *P. aeruginosa* to inhibit the biofilm formation of *C. albicans* by suppressing the hyphae formation of *C. albicans* and eventually inducing death by secreting neurotransmitters [7]. The results showed that *E. coli* inhibits *C. albicans* more than gram-positive bacteria (*S. aureus* and *S. pyogenes*). Strong inhibition of *C. albicans* biofilm formation by *E. coli* in the intestines [11]. This inhibitory effect of *C. albicans* is not limited to the living cells of bacteria, but rather to heat-treated bacteria, where biofilm formation of *C. albicans* was reduced when incubated with heat-treated bacteria [12].

Transcriptase real-time PCR

The study performed quantitative reverse transcriptase PCR to assess the expression of hyphae-specific genes during the biofilm development of *C. albicans*. Over time, the expression of hyphae-specific genes (*Sap5* and *Ece1*) was strongly enhanced (Figure 2)

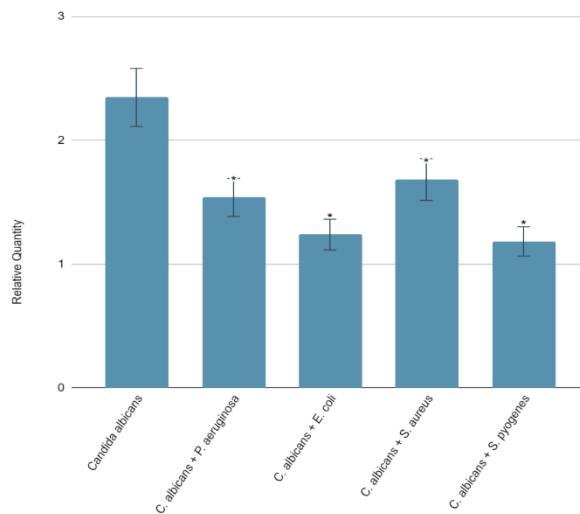


**Figure 2:** The relative expression of genes responsible for

hyphal development (*Ece1* and *Sap5*) by using reverse transcription real-time PCR in *C. albicans* during biofilm experiment for 24 and 48hrs. The results show the values of mean  $\pm$ Stand Dev. of separate *C. albicans* cultures, statistically significant was  $p < 0.05$  (\* $p < 0.05$ , \*\* $p < 0.01$ ).

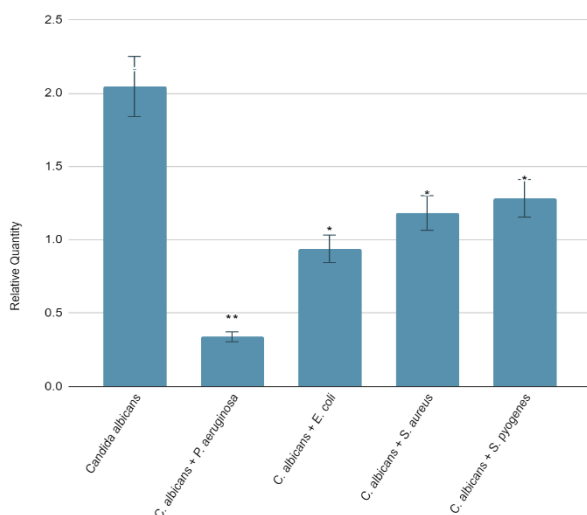
To analyze the inhibition effects of *C. albicans* and bacteria mixed cultures on biofilm experiments included mRNA quantitatively of hyphal specific genes was. The expression levels of the two genes (*Ece1* and *Sap5*) of *C. albicans* in biofilm mixed cultures with bacteria (*P. aeruginosa*, *E.coli*, *S. aureus* and *S. pyogenes*).

The expression level of *Ece1* gene when *C. albicans* mixed with *E.coli* and *S. pyogenes* showed decreased *Ece1* mRNA expression compared with *P. aeruginosa* and *S. aureus*. This was the substantial reduction of mRNA expression of hyphal developed genes when *C. albicans* mixed with *E.coli* and *S. pyogenes* for 24 hrs of biofilm experiment (Figure 3).



**Figure 3:** The relative expression of *Ece1* gene was determined by using reverse transcription real-time PCR in mixed culture *C. albicans* with bacteria for 24 hrs. Beta-actin (housekeeping) gene was used for normalization of amplification reactions. The results show the values of the mean  $\pm$ Stand Dev. of separate *C. albicans*, statistically decreased gene expression is significant at  $p < 0.05$  (\* $p < 0.05$ , \*\* $p < 0.01$ ).

The mRNA levels of *Sap5* hyphal developed gene were decreased by *C. albicans*, the mRNA level of *Sap5* gene was significantly reduced by *C. albicans* mixed with *P. aeruginosa* than *E.coli*, *S. aureus*, and *S. pyogenes*. This was the most significant reduction of mRNA expression of hyphal developed genes when *C. albicans* cultured with *P. aeruginosa* (Figure 4).



**Figure 4:** The relative expression of Sap5 gene was determined by using reverse transcription real-time PCR in mixed culture *C. albicans* with bacteria for 24 hrs. Beta-actin (housekeeping) gene was used for normalization of amplification reactions. The results show the values of the mean  $\pm$ Stand Dev. of separate *C. albicans*, statistically decreased Sap5 gene expression is significant at  $p < 0.05$  (\* $p < 0.05$ , \*\* $p < 0.01$ ).

In this study, *C. albicans* and *P. aeruginosa* co-cultured together showed inhibition of biofilm formation of *C. albicans*, consistent with the study reported of the ability of *P. aeruginosa* to inhibit the biofilm formation of *C. albicans* by suppressing the hyphae formation of *C. albicans* and eventually inducing death by secreting neurotransmitters [7]. Also in the previous study, *E. coli* was able to suppress biofilm formation of *C. albicans* by inhibiting adhering to an extracellular surface [8]. Although the biofilm of *C. albicans* is inhibited by bacteria, the mere presence of *C. albicans* does not have an effect on bacterial biofilm formation. *C. albicans* biofilm is formed through many steps and has a complex structure, while bacterial biofilms have more simplistic structures during the biofilm formation process [12]. Moreover, the expression of Ece1 was correlated with *Candida* hyphal elongation [13]. Sap5 was found to be expressed on biofilm-associated mucosal surfaces [14]. These genes were up-regulated as biofilm formation progressed. Interestingly, expression of the *Candida* genes was significantly inhibited by co-culturing with *P. aeruginosa*, *S. pyogenes*, and *E. coli* in this study.

Interestingly, gram-negative bacteria are motile. The opposite of gram-positive bacteria is non-motile. In particular, with the highest motile activity [15], remarkably inhibited the biofilm formation of *C. albicans*. These results correspond to the mRNA expression levels of hyphae-specific genes. This suggests that gram-negative cell wall constitutions and/or motile activity of bacteria may inhibit the biofilm formation of *C. albicans*.

## Conclusion

The findings showed that structural discordance between bacteria and *C. albicans* may impede the growth of the latter's biofilm. The down-regulation of hyphal transition-associated gene expression may be influenced by bacterial growth. Future research should also focus on determining the elements that influence biofilm development as a result of the interaction between the fungus *C. albicans* and various bacterial species.

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