Determination the Gene Expression Levels of adhesins and Extracellular Enzymes Genes in *Candida albicans* biofilm producer by Quantitative Real Time PCR Technique (qRT-PCR)

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Abstract

In the present study, all *C. albicans* were biofilm producers with variable strength. Molecular study of virulence genes correlated with biofilm *C. albicans* showed that *HWP1*, *ALS1*, *ALS3*, *SAP5*, *PLB1* and *LIP8* genes were detected in 100% of *C. albicans* from vaginal and oral infections. In addition to that quantitative real time PCR technique show that *ITS1* was used as the housekeeping gene and was shown to be stably expressed throughout all biofilm conditions and *HWP1*, *ALS1*, *ALS3*, *SAP5*, *PLB1* and *LIP8* where 100% expressed in all ten tested *C. albicans*. It was appeared that adhesins genes such as *HWP1*, *ALS1* and *ALS3* were highly expressed in HBF and less expressed in LBF, whereas in very low BF producers the fold up-regulation were not particularly high, but they were over expressed in strong biofilm producers in both of vaginal and oral isolates. The average of gene expression folding show that *SAP5* and *PLB1* were highly expressed among vaginal isolates rather than oral ones, in contrast, *ALS1*, *HWP1* and *LIP8* were predominant expressed among oral isolates, whereas *ALS3* was higher in vaginal isolates than oral with little differences.

Gene expression levels by q RT-PCR show that *SAP5* and *PLB1* were up-regulated in both of HBF and LBF producers, and they were highly expressed among the ten tested isolates. The over expressions of *SAP5* and *PLB1* genes were observed in vaginal isolates with high biofilm. The current study found an overexpression of adhesins such as *HWP1*, *ALS1*, *ALS3*, are responsible in strength of biofilm formation in *C. albicans* isolated from oral and vaginal infection with different ages and different clinical states, in addition to upregulated of extracellular hydrolytic enzymes genes belong to *SAP*, *PLB* and *LIP* was important in biofilm formation.

Key words: *Candida albicans, biofilm, ALS1, ALS3, HWP1, SAP5 , PLB1, LIP, gene expression.*

Introduction

In the recent years, the prevalence of serious fungal infections, invasive *Candida* infections particularly, has been increasing due to an increased number of patients receiving immunosuppressive therapy, increased major surgeries and broad-spectrum antibiotherapy, hyperalimentation, prolonged intensive care unit stay for patients with poor health status[1,2].

Candida albicans is responsible for more than 50% of human candidiasis, including two major types of infections, superficial infections (nonlethal), such as oral or vaginal candidiasis; and systemic infections [3,4]. *Candida* spp. is the most causative agents of vulvovaginitis in women and *C. albicans* was the most predominant *Candida* spp.[5].

The medical impact of *C. albicans* (like that of many other microorganisms) depends on its ability to grow up as a biofilm, a closely packed community of cells [6]. Microbial adhesion is considered the first step for biofilm formation. This structure constitutes a protective milieu against environmental stresses and

human host defenses[7]. *C. albicans* has a specialized set of proteins (adhesins) which mediate adherence to other *C. albicans* cells to other microorganisms, to abiotic surfaces and to host cells [8]. *C. albicans* adhesions are the agglutinin-like sequence (*ALS*) proteins which form a family consisting of eight members (*ALS*1–7 and *ALS*9). Another important adhesin of *C. albicans* is *Hwp1*, which is a hypha-associated protein [9]. An essential factor to the virulence of the genus *Candida* is the ability to produce enzymes and this may be crucial in the establishment of fungal infections [10],

There are two major families of histolytic enzymes produced by *Candida* species: the secretory aspartyl proteinases (*SAP*) and phospholipases (*PL*) [11]. The secreted aspartic proteases (Saps) are among the most studied virulence determinants in *C. albicans* [12].

Many biofilm genes encode known or predicted cell wall proteins. These proteins are of special interest because they may play a direct role in cell-substrate or cell-cell adherence, heterologous expression studies indicate that *HWP1, ALS1,* and *ALS3* have such roles [13]. Previous works demonstrated that the expression of *HWP1* and of genes belonging to the *ALS*, *SAP, LIP* and *PLB* gene families is associated with biofilm growth on mucosal surfaces [14], In addition to *SAPs*, *C. albicans* also has two other gene families, namely the lipases (*LIP*) and phospholipases (*PL*) that produce extracellular hydrolytic enzymes that could play roles in candidal adhesion, nutrient acquisition and invasion of epithelial surfaces [15]. Constitutive expression of the *LIP* genes and *PLB* has been demonstrated in *C. albicans* biofilms [16], However, it is known that the expression of *ALS, SAP, LIP and PLB* genes can be influenced by other factors such as the growth medium, temperature and other environmental conditions [17, 18]

Materials and Methods

Samples collection and isolation

High vaginal swabs were collected from 49 patients aged between16-50 years, presented with vulvovaginal candidiasis. Oral swabs were collected from 47 patients with oral candidiasis aged between two days to ten years, during the period from March 2015 to the end of June 2015. Clinical presentations were done by specialized doctors. and were divided in to two smears: one smear was examined immediately under microscope for direct examination; the other usually was cultured on SDA medium.

Identification of Candidal Isolates*:*

the isolates were purified by streaking on sabouraud dextrose agar by using ABC methods then incubated at 37ºC for two days to obtained one isolated pure colony. This isolated colony was transferred to SDA by streaking all the plate, and then incubates at 37 ºC over night

Candida was identified depending on the morphological features on culture medium and germ tube formation conferring the diagnosis by identifying *Candida* Spp By using Vitek 2 system.

Biofilm Formation Assay

In the present study, biofilm formation was determined using pre-sterilized polystyrene 96-well microplates using method described by Melek *et al*. (2012) [20] with modifications. Yeast was inoculated using a loop into tube containing 2 ml of YPD broth incubated at 37°C for 24 h, all tubes were diluted at a ratio of 1:20 by using freshly prepared YPD with 1% glucose, each well of the microplate was filled with 200 µL of this final solution. Microplates were covered with lids and incubated at 37°C for 24 h.

The medium in wells was removed and washed two times with sterile phosphated buffer solution (PBS) and then inverted to blot and let to dry. Microplates were stained by adding 200 µL of 0.1gm/100ml crystal violate to each well incubated for 20 minutes which then washed two times with PBS then inverted to blot and let to dry, finally 200 µL of acetone: ethanol mixture (20:80 v/v) was added to each well, waiting for about 10 min then the results were read at 450nm by an Elisa reader.

Gene Expression Study

Gene expression level for *ALS*1, *ALS*3, *HWP*1, *LIP*8, *SAP*5, and *PLB*1 gene were determined by comparative Ct method to measure the level of gene transcription (mRNA level). The Ct of ITS4 was used as endogenous control for calibrator the Ct values of other genes.

The folding of gene expression was calculated as mentioned bellow:

* Ct= cycle of threshold (number of cycles required for florescent signal

to cross threshold).

* HK= housekeeping gene.

Primers selection and preparation

All primers (table1) were supplied in lyophilized forms. Dissolved in nuclease-free water to give a final concentration of 100 picomol⁄µl as recommended by provider and stored in deep freezer as stock solution until used in PCR amplification. Work solution was prepared by added 90 µl of nuclease free water to 10 µl of stock solution of primer to get 10picomol/ µl concentration.

Isolation of RNA from Yeast:

1) Yeast was cultured on YPD broth for 20 h at 37, the culture was diluted 1:50 and grow until the OD600 is 0.6–1.0. This should only take a few hours.

2) The culture was centrifuged for 2 minutes at 14,000 rpm in a microcentrifuge.

3) The pellet was resuspended in 100µl of the following solution:

· 1M sorbitol

· 0.1M EDTA (pH 7.4)

· Just before use 0.1% β-mercaptoethanol and 50 units of lyticase were added

4) Incubate at 30°C for 15–30 minutes until the solution appears clear.

5) Seventy-five microliter of RNA Lysis Buffer was added, Mix gently.

6) Three hundred-fifty microliter of RNA Dilution Buffer (blue) was added, and Mixed by inversion and centrifuge at maximum speed for 10 minutes in a microcentrifuge.

RNA purification by Centrifugation Method:

1) The cleared cell lysate solution was transferred to a microcentrifuge tube and 200 µl of 95% ethanol were added, then the solution was mixed by pipetting several times.

2) The mixture was transferred to the spin column assembly and centrifuged at 13,000 rpm for 3 minutes.

3) Spin basket was removed from the spin column assembly, and the liquid in the collection tube was discarded; the spin basket was again put back into the collection tube.

4) Six hundred microliters of RNA wash solution was added to the spin column assembly and centrifuged at 13000 rpm for 3 minutes.

5) DNase incubation mix were prepared by combining 40µl yellow core buffer, 5 µl 0.09 M $MnCl_2$ and 5µl of DNase I enzyme per sample in a sterile tube mix by gentle pipetting.

6) Fifty microliters of this freshly prepared DNase incubation mix were applied directly to the membrane inside the Spin Basket and incubated for 15 minutes at 25°C.

7) Two hundred microliters of DNase stop solution were added to the spin basket, and centrifuged at 13000 rpm for 5 minute.

8) Six hundred microliters of RNA wash solution (with ethanol added) were added and centrifuge at 13000 rpm for 5 minute.

9) The collection tube was emptied, added 250µl RNA wash solution (with ethanol added); and centrifuged at 13000 rpm for 5 minutes.

10) Twisting motion was used to remove the cap from the spin basket, and transferred spin basket from the collection tube to the1.5ml elution tube.

11) One hundred microliters of nuclease free water were added to the membrane, and placed the spin basket assemblies in the centrifuge with the lids of the elution tubes facing out, and centrifuged at 13000 rpm for 5 minutes.

12) The spin basket was discard, then capped the elution tube containing the purified RNA and stored at -70°C.

RNA Concentration and purity Determination

Quantus Florometer was used to detect the concentration of extracted RNA in order to detect the

goodness of samples for downstream applications. For 1 µl of RNA, 199 µl of diluted QuantyFlour Dye was mixed. After 5min incubation at room temperature, RNA concentration values were detected.

Quantitative Real Time PCR (qRT–PCR)

The expression levels of *ALS1*, *ALS3, HWP1*, *LIP8*, *SAP5*, and *PLB1*, were estimated by One Step qRT-PCR. To confirm the expression of target gene, quantitative real time one step qRT-PCR sybr Green assay was used. Primers sequences for each gene were prepared. The mRNA levels of endogenous control gene ITS4 were amplified and used to normalize the mRNA levels of the up genes.

Statistical Analysis

The Statistical Analysis System- SAS (2012) program was used to effect of difference factors in study parameters. Chi-square test was used to significant compare between percentage in this study.

Results and Discussion

From 49 *Candida* isolates from vaginal swabs; 22(45%) were *Candida albicans* and 27(55%) were non *albicans*. Whereas 22(47%) out of 47 *Candida* isolates from oral swabs were *C. albicans* and 25(53%) were non *albicans*.

In the present study, non *albicans Candida* isolates collectively contributed to more than half (55%) and (53%) of the candidial infections in both of vaginal and oral infection respectively this result agree with Jose *et al*. [24] in a previous study, also observed that the non *albicans Candida* was predominant (70%) as compared to *C. albicans* (30%), which indicate that the non a*lbicans Candida* infections are on the rise.

Similar finding have been reported in the literature by different authors [25], Whereas *C. albicans* was the most dominant species in both of vaginal and oral infections with percentages 45% and 47% respectively. These results are agree with [26] that *C. albicans* and *C. glabrata* were the most common yeast species isolated from patients. Mohammed [5] indicated that *C. albicans* was the predominant species (63.6%) out of 124 HVS, followed by *C. glabrata* (30.9%) and *C. trupicalis* (5.5%), [27] find 63.8% isolates were *C. parapsilosis*20.34% were *C. albicans*.

Biofilm Formation of *C. albicans*

At the present study; all *C. albicans* were biofilm producers with variable strength value depended on the OD value with using crystal violate (fig.1). In general out of 44 BF producers, 18 (40.9%) were weak BF (low biofilm) with significant differences ($P<0.05$) between oral and vaginal specimens, 25 (56.8%) moderate BF (high) and just one isolate from oral was strong (very high) biofilm in percentage about 2.3%. Among vaginal isolates; 10/22 (45.5%) were low BF whereas moderate or high BF were 12/22 (54.5%). In comparable with those; the biofilm among oral isolates was higher than those of vagina, that weak BF were produced by 8/22 (36.4%), moderate BF 13/22 (59.1%) in addition to the appearance of one oral isolates produced strong BF in percentage about 4.5% (table 4).

As any other research these results are agree with some and different with others in some sides. These results show that BF is higher among oral infection than vaginal infection. These results agree with Mahmoudabadi *et al*. [28] indicated that 100% of *C. albicans* isolated from different sources had the ability to produce biofilm *in vitro*. It also agreed with Villar-Vidal *et al*.[29] found all tested isolates of *C. albicans* produced biofilm on polystyrene.

Bruder-Nascimento *et al*. [30] found that total of 198 of 327 (60.6%) *Candida* species isolates were biofilm-positive. Of these, 72 (36.4%) and 126 (63.6%) isolates were low and high biofilm producers, respectively.

Udayalaxmi *et al*. [31] reported that among 40 *C. albicans*, 22(55%) were strong- moderate biofilm producers. which agree with current results. Jose and colleagues [24] found that Among the 100 isolates of *Candida*, 69% were found to be biofilm producers. Among them, 42% were weakly adherent, 23% moderately adherent, and 4% were strongly adherent.

Determination Gene Expression Level by using Quantitative Real Time PCR Technique (qRT-PCR):

Gene expression level is usually used to identifying the important genes involved in *Candida albicans*

biofilm formation. In the present study, gene expression were studied for ten candidal isolates chosen randomly which five isolates from vulvovaginal candidiasis and other five from oral candidiasis according to their biofilm formation. These isolates were termed by numbers from one to five for vulvovaginal candidiasis isolates, from six to ten for oral candidiasis isolates; respectively. The immediately isolated purified RNA from each isolates was used to determine the expression of the goal genes (*ALS1*, *ALS3*, *HWP1*, *SAP5*, *PLB1* and *LIP8*) involve in biofilm formation of *C. albicans*. The expression level for each gene was calibrated and normalized with housekeeping gene of *C. albicans* internal transcription spacer sequence (*ITS*1).The gene expression values of housekeeping gene, tested genes and Delta Ct values. The average of gene expression folding that *SAP5* and *PLB1* were highly expressed among vaginal isolates rather than oral ones, in contrast, *ALS1*, *HWP1* and *LIP8* were predominant expressed among oral isolates, whereas *ALS3* was higher in vaginal isolates than oral with little differences (fig. 2).

Expression Levels of *HWP1* and *ALS* Genes:

C. albicans clinical isolates defined as LBF and HBF were further assessed at a transcriptional level and the expression of genes related to biofilm formation was investigated. *ITS1* was used as the housekeeping gene and was shown to be stably expressed throughout all biofilm conditions.

The present results show that *HWP1*, *ALS1*and *ALS3* genes were expressed in all tested isolates for both of oral and vaginal candidiasis. The expression levels show that these genes were up-regulated in biofilm (BF) formation isolates with highest expression folding was 1.662, 1.176, 0.630 for *ALS1*, *ALS3* and *HWP1* respectively; whereas the lowest expression folding was 0.901, 0.370, 0.210 for *ALS1*, *ALS3* and *HWP1* respectively.

The majority of the genes tested followed a trend up-regulation in HBF compared to LBF. However It was appeared that *HWP1*, *ALS1* and *ALS3* were highly expressed in high biofilm HBFand less expressed in low biofilm, whereas in very low BF producers the fold up-regulation were not particularly high, but they were over expressed in strong (very high) biofilm producers in both of vaginal and oral isolates. In staid of that *HWP1*, *ALS1* and *ALS3* were predominant highly expressed levels among biofilm producers of oral isolates. Microbial adhesion is considered the first step for biofilm formation. This structure constitutes a protective milieu against environmental stresses and human host defenses [7].

these results are agree with Rajendran *et al*. [32] that the expression of biofilm-related genes *HWP1* and *ALS3* in *C. albicans* biofilms was evaluated and it was found that these were up-regulated in isolates with high biofilm. Despite *ALS3* and *HWP1* expression being increased in high BF when compared to low BF. In another recent study of Rajendran *et al*. [33] which improved their previous study; that high BF had hyphal specific gene expression increased *HWP1* and *ALS3*, Similar to the biofilm formation process, there was no difference in the percentage of up-regulated genes associated with cell adhesion and hyphal growth in the LBF and HBF, which in total accounted for only 4% of up-regulated genes in each group.

The expression of *ALS1*, *ALS3* and *HWP1* has already been observed in biofilms associated with abiotic surfaces [34]

Garcia-Sanchez *et al*. [35] found that the *ALS1* gene was clearly up-regulated in biofilms when compared to planktonic cells which can explain the high expression of *ALS1* in LBF (fig.3). The case of *C. albicans*, *ALS3* appears to play a key role in adhesion to oral epithelial cells, and it is also related to the extent of subsequent epithelial damage and induction of epithelial cytokines[36], an overexpression of *ALS3* was observed in initial stages of biofilm formation [34], in study by Nailis *et al*.,reported that overexpression of *ALS3* and *HWP1* were more pronounced in biofilms grown in the *in vivo* model, and their expression levels were higher in the catheter reactor than in the microtiter plate (MTP) and this may explain the up-regulation of *HWP1*and *ALS* genes in some isolates with very low biofilm producers [37].

Hu *et al*. [38] found 16 high biofilm formers from a total of 104 clinical isolates. Although there were two strains showing higher expression of *HWP1* and four different strains showing higher expression of *ALS3*, their up-regulation did not directly correlate with the ability to form abundant biofilms. The present results of

gene expression found that *HWP1* was the most affected gene in HBF fallowed by *ALS3*, whereas *ALS1* have less affect in HBF, that some isolates with LBF show particularly high up-regulation of these genes.

Furthermore, *ALS3* and *HWP1* were highly up-regulated in biofilms grown in the reconstituted human epithelium (RHE) model, Nailis *et al*., [37] established an increase in the percentage of filaments during biofilm formation in this model system. *C. albicans* needs to invade and destroy epithelial cells, in order to grow in the RHE model, and hyphae are known for their increased invasiveness [14] which is not surprising as hyphae are the predominant form in biofilms grown in this model system due to the overexpression of *HWP1* and *ALS* genes [39]

The *ALS* genes aggregate becomes in essence a multivalent adhesin. Thus, even weakly bound ligands are rebound rapidly after they are released [40], which explain the overexpression of adhesins among strong and moderate (HBF) biofilm in this study.

The initial stage of biofilm is adherence, and how is adherence regulated? As mentioned above, many of the major known adhesins are expressed at the highest levels on hyphal cells. Their expression is regulated by transcription factors that also govern hyphal development [41]

It has been suggested that *HWP1* surface protein requires *ALS1/3* association to initiate *in vivo* biofilm formation [42], a complementary role was suggested for *ALS1/3* and *HWP1* genes in *in vivo* and *in vitro* biofilm formation [43]. The present result is consistent with reports showing that the combined expression of the adhesion proteins *HWP1* and *ALS1–ALS3* significantly facilitates biofilm formation. The high frequency of expression of *HWP1* and the *ALS1/3* genes found in this study reveals the capacity of the isolated strains to cause chronic vaginal and oral infections.

Expression Levels of *SAP5, PLB1* and *LIP8* Genes in *C. albicans*:

The present study showed that not only *ALS1, ALS3* and *HWP1*, but *SAP5*, *PLB1* and *LIP8* gene families were also expressed in biofilm; the current results demonstrated that genes encoding adhesins and genes encoding extracellular hydrolases are constitutively expressed in biofilms grown on abiotic surfaces *in vitro*.

qRT-PCR analysis shows that variant expression levels of *SAP5*, *PLB1* and *LIP8* in *C. albicans* high biofilm producers isolates in staid of low biofilm produce, the highest expression folding was 6.727, 2.462, 0.637 for *SAP5*, *PLB1* and *LIP8* respectively; the lowest expression folding was 1.447, 0.724, 0.366 for *SAP5*, *PLB1* and *LIP8* respectively.

In general; gene expression level show that *SAP5* and *PLB1* were up-regulated in both of HBF and LBF producers, and they were highly expressed among the ten tested isolates (fig.3).

C. albicans can generate a number of hydrolytic enzymes with broad substrate activity that can damage host cell structures. Perhaps, the most extensively studied extracellular hydrolytic enzymes of *C. albicans* are the secreted aspartyl proteinases [45] Previous research demonstrated that members of the *SAP* gene family are expressed in biofilms associated with mucosal surfaces [46]. the present results investigated that *SAP5* was also highly expressed in biofilm associated with abiotic surfaces. Mendes *et al*. [47] showed that *C. albicans* biofilms secrete more *SAPs* than do planktonic cells, while Ramage *et al*. [48] showed that an *in vitro C. albicans* biofilm induced *SAP* activity, and that *SAP8* expression within the biofilm correlated with *in vivo* denture stomatitis severity.

Results presented by Monroy-Pérez *et al*. [49] showed that all of the *SAP* genes were expressed in the reconstituted human vaginal epithelium (RHVE), suggesting that the Sap proteins play an important role in the pathogenesis of infection.

Of the 10 *SAP*s, expression of *SAP4, 5* and *6* has been demonstrated in all mucosal and systemic infections by *C. albicans* examined [46]. In addition, *C. albicans* expresses the *SAP4 – 6* ubiquitously in all *Candida* carriers and patients with oral and vaginal candidiasis, regardless of the infection model, suggesting that this proteinase subfamily plays an important role in *C. albicans* colonization and infection [18].

Li and colleagues [50] suggested that *Sap2* and *Sap5* were the most common genes expressed during oral mucosal infection and with *Sap5* and *Sap9* most strongly expressed throughout the course of infection.

Table 1: The primers and their sequences used in conventional PCR technique

Primers	Primers sequences (3`-5`) Forward reverse	Annealing temperature (°C)	Product size (bp)	Reference
ALS1	GAC TAG TGA ACC AAC AAA TAC CAG A CCA GAA GAA ACA GCA GGT GA	52	318	21
ALS3	CCA CTT CAC AAT CCC CAT C CAG CAG TAG TAG TAA CAG TAG TAG TTT CAT C	58	342	22
HWP1	ATG ACT CCA GCT GGT TC TAG ATC AAG AAT GCA GC	52	572	21
LIP8	AGA GTG ATA CAG ACA AAA AAT CAG AAG ACC ATT CAG CAT CAT GGT G	59	521	15
SAP5	AGA ATT TCC CGT CGA TGA GAC TGGT CAA ATT TTG GGA AGT GCG GGA AGA	60	277	23
PLB1	CCT ATT GCC AAA CAA GCA TTG TC CCA AGC TAC TGA TTT CAC CTG CTC C	58	179	23

Table 2: Reaction volume and components of RT qPCR:

Components	Conc.	Volume(µl)\Reaction
GoTaq qPCR master mix	2X	10 µl
RT mix		0.4 µl
Forward Primer	10µM	2 µl
Revers Primer	10µM	2 µl
RNA	1-2ng	4 µl
RNase-free water	-	1.6 µl
Total per reaction	20 µl	

Table 3: Thermal Cycler Programming:

Steps	C°	min:sec	Cycles
cDNA Synthesis	37	15min	1
Initial Denaturation	95 C°	5 min	1
Denaturation	95 C°	30 sec	40
Annealing	60 C°	30 sec	
Extension	72 C°	30 sec	
Melt	65-90 C°		1

Table 4: Biofilm formation by *C. albicans*

Candida albicans source	Biofilm Strength ODc= 0.060 No. (%)		
	Weak BF	Moderate	Strong
VVC (n= 22)	10 (45.5%)	12 (54.5%)	0
OC (n= 22)	8 (36.4%)	13 (59.1%)	1 (4.5%)
Total (n= 44)	18 (40.9%)	25 (56.8%)	1 (2.3%)
Chi-square –χ2	4.367 *	2.159 NS	1.077 NS
P-value	0.0488	0.148	0.375

* (P<0.05), NS= Non-significant.

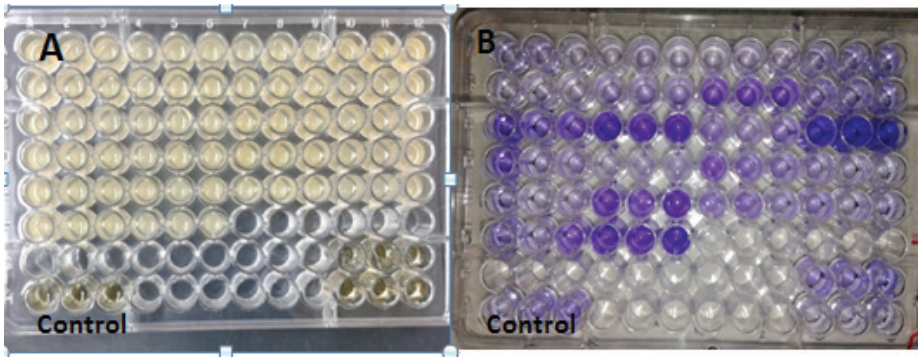

Figure1: Microtiter plate for biofilm formation, *Candida albicans* isolates biofilms after 24h at 37°C cultured on YPD with 2% glucose. A) before staining B) after staining with 0.5 mg/100ml crystal violate.

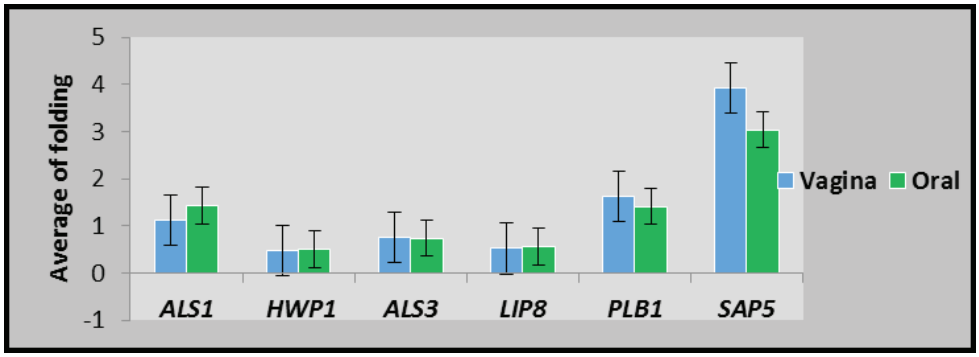

Figure 2: Averages of expression folding of *ALS1*, *ALS3*, *HWP1*, *SAP5*, *LIP8* and *PLB1* of *C. albicans* among oral and vaginal isolates.

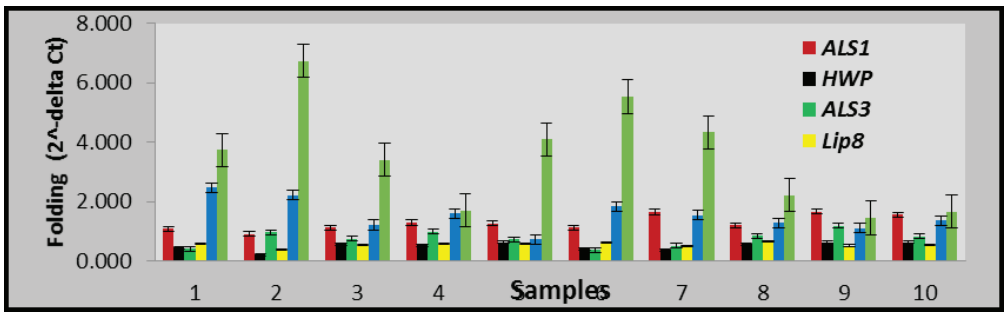

Figure 3: Gene Expression by qRT- PCR Technique of *ALS1*, *ALS3*, *HWP1*, *SAP5*, *PLB1* and *LIP8* Affected in Biofilm Formation of *Candida albicans*, Samples 1-5 Isolated from Vulvovaginal Candidiasis, from 6-10 Isolated from Oral Candidiasis.

Conclusions

1. Quantitative real time PCR technique show that *ITS1* was used as the housekeeping gene and was shown to be stably expressed throughout all biofilm conditions and *HWP1*, *ALS1*, *ALS3*, *SAP5*, *PLB1* and *LIP8* where 100% expressed in all ten tested *C. albicans*.

2. Adhesins such as *HWP1*, *ALS1* and *ALS3* were highly expressed in HBF and less expressed in LBF, whereas in very low BF producers the fold up-regulation were not particularly high, but they were over expressed in strong biofilm producers in both of vaginal and oral isolates.

3. *SAP5* and *PLB1* were highly expressed among vaginal isolates rather than oral ones; in contrast, *ALS1*, *HWP1* and *LIP8* were predominant expressed among oral isolates, whereas *ALS3* was higher in vaginal isolates than oral with little differences.

4. The overexpression of adhesins such as *HWP1*, *ALS1*, *ALS3*, are responsible in strength of biofilm formation in *C. albicans* isolated from oral and vaginal infection with different ages and different clinical states, in addition to upregulated of extracellular hydrolytic enzymes genes belong to *SAP*, *PLB* and *LIP* were important in biofilm formation.

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Bilad AlRafidain University Collage Dyala and all experiments were carried out in accordance with approved guidelines.

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