

The Effect of Growth Condition on SAP10 Gene Expression in Genotype A *Candida albicans*

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Abstract:

There are a limited data regarding to the role of the growth conditions on pathogenicity process of *Candida albicans* and if the host play a role in augmenting the virulence of this microorganism, this study focusing on the effect of environmental growth conditions of genotype A *Candida albicans* on SAP10 gene expression as one of the Secreted Aspartyl Proteinase super family genes that play a main role in *Candida albicans* pathogenicity process.

Ten pathogenic strains of *Candida albicans* obtained from clinical cases were included in this study, genotyped according to 25s rDNA and grown on two different condition using RPMI1640 medium at 37C□ for mimic host condition (under controlled condition in vitro) and also grown on Sabauroud Dextrose agar at 25C□ as in vitro condition, total RNA were extracted from each condition and evaluated using Pfaffle's equation.

The results of this study exhibit that all tested isolates classified under genotype (A) *Candida albicans* with 450pb PCR product size of 25s rDNA, while SAP 10 gene expression data indicate that no significant expression pattern related to the different growth conditions and the expressions are related to the tested strains and no relation between *Candida albicans* growth conditions, strain genotype and SAP10 gene expression is approved in this study.

Key words: *Candida albicans*, SAP10, Genotyping, growth condition.

تأثير ظروف النمو على التعبير الجيني لجين (SAP10) في النمط الجيني A في لمبيضات البيض

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الخلاصة:

هناك بيانات محدودة فيما يتعلق بدور ظروف النمو في عملية امراضه خمائر المبيضات البيض ، وإذا كان المضيف يلعب دورا في زيادة فوعة هذه الكائنات الدقيقة ، ركزت هذه الدراسة على تأثير ظروف النمو البيئية للنمط الوراثي A للمبيضات البيض على تعبير الجين SAP10 كواحد من جينات عائلة SAP التي تلعب دوراً رئيسياً في امراضه المبيضات البيض.

تم تضمين عشر سلالات ممرضة من المبيضات البيض تم الحصول عليها من حالات السريرية في هذه الدراسة ، صنفت وراثيا وفقا لـ 25s rDNA ونمت على وسطين مختلفين باستخدام وسط RPMI1640 عند 37 درجة مئوية لمحاكاة حالة المضيف (تحت ظروف التحكم في المختبر) وأيضا نمت على أجار سابورودي في درجة حرارة 25 كما في حالة المختبر ، تم استخراج الحمض النووي الريبي من كل حالة وتقييمها باستخدام معادلة Pfaffle.

تظهر نتائج هذه الدراسة أن جميع العزلات المختبرة مصنفة تحت النمط الجيني (A) *Candida albicans* مع

حجم منتج PCR بمقدار 450 pb من 25s rDNA ، بينما تشير بيانات التعبير الجيني SAP 10 إلى عدم وجود نمط تعبير معنوي مرتبط بظروف النمو المختلفة والتعبيرات المرتبطة إلى السلالات التي تم اختبارها وعدم وجود علاقة بين ظروف نمو المبيضات البيض، والنمط الجيني لسلالة المبيضات البيض والتعبير الجيني SAP10 في هذه الدراسة.

Introduction:

Candida albicans is the one of most important fungal pathogens causing infections in all populations in the world[1],[2] in spite of the fact that the most of these clinical infections are recovered spontaneously but still many of them consider as a fatal especially in the immunocompromized peoples, and with time the medical important of this microorganism increased [3][4], As an opportunistic microorganism, the predisposing determinants and virulence factors of *Candida albicans* considered as the key point in the pathogenesis of this microorganism, these factors restricted in the following aspects : morphological switching, tissue invasion, biofilm formation and enzymes production.[5]

Several studies have been conducted on the role of aspartyl protease production in addition to the other hydrolysis enzymes in the pathogenicity process of *Candida* species through digesting the host epithelial cells[6] and also several studies approved that presence of group of genes (super family genes) responsible for production of this proteins in *Candida albicans*[7][8].

Aspartyl protease produced from pre-pre-enzymes composed of 60 amino acid configure in 3D as two aspartate residues and 4 cysteine residues in addition to the N- terminal [9], the active form translated directly from mRNA, and it lose the n-terminal peptide under effects of Kex2 enzyme[10] on its way through endoplasmic reticulum and then Golgi apparatus and then to secretory vesicles which attached to the cell membrane[11]the regulation of the production of this protein is not clearly identified but it is related in general

with mRNA level expression of group of 10 genes (SAP genes family) [7][8] and this related with many factors most of them still obscure till now.

The effects of environment condition on *Candida albicans* behavior are detected previously but still the effect of the environment on pathogenicity process is not clear and still the question that "if *Candida albicans* is pathogenic by itself or due to response to some environmental factors" that determined the behavior of this microorganism in the host tissue. this study focusing on the effect of in vivo condition and genotyping of *Candida albicans* regarding to 25s rDNA on SAP10 gene expression by using RPMI1640 medium to mimic the host condition under control condition (in vitro) and distinguish if the host environment play a role in *Candida albicans* pathogenicity using SAP10 gene as indicator.

Method:

Genotyping analysis:

This study was carried out on a group of ten *Candida albicans* strains obtained from College of Veterinary Medicine \ University of Kufa, all strains cultured on CHROMagar medium to detect the characteristic colony shape and color and then tested to produce the germ tubes, the strains were cultured overnight in 10ml of SAB broth, centrifuged and washed twice with grade water, DNA-Pure Yeast Genomic kit (bioWorld, USA) was used for genome extraction and purification following manufacturer's instructions. Genotyping of study strains were conducted by using PCR technique and specific primers (PI and PII) [Table 1] for 25s intron detection according to Mccullough et. al. procedures. [12]

Secreted Aspartyl Proteinase 10 (SAP10) gene expression analysis:

All strains were cultured on Sabraud dextrose agar (SAB) overnight at 25 C□ and then cultured on two different condition: laboratory conditions (on SAB at 25C□) and host conditions (on RPMI1640 at 37C□), for 12 hours; the strains were harvested by centrifugation and washed twice and prepared for next steps. RNA extracted was done by using Yea™ Star RNA Kit from (ZYMO RESEARCH, USA). The genomic RNA converted to cDNA by using cDNA Synthesis Kit from (YEKTA TAJHIZ AZMA, IRAN) regarding to the manufacture's instruction and stored at -80 in the deep freezer for the next steps.

Primers from Samaranayake et al.[13] were used for analysis SAP10

gene expression; and ACT1 from [14] was used as reference gene in the quantification real-time PCR experiments using Pfafell's equation [15]; Each sample was test twice and the mean was used for data analysis by MxPro QPCR software from (Agilent, USA). The following protocol was used in the (SAP10) gene expression in the real time PCR system (MX 3005P system, Agilent, USA) using KAPA SYBR FAST qPCR kit instruction (Kapabiosystems Co., South Africa) as follow: 95C° for 3 minutes following by 40 reaction cycles as follow : 95C° for3 sec , 60 C° for 34 sec and 72C° for 30 sec , finally dissociation curve as instrument default :95C° for 1 minutes , 55C° for 30 sec , and 95C° for 30 sec .

Table 1: Forward and reverse primers used in this study for testing the quantification the expression of *C. albicans* SAP10 gene.

primers		Primers sequence
25s rDNA	PI	ATA AGG GAA GTC GGC AAA ATA CCG TAA
	PII	CCT TGG CTG TGG TTT CGC TAG ATA GTA GAT
SAP10	SAP10R	CCG TCC TTT TCA GTC TTG AGA TC
	SAP10F	GGT TTT CGA TAG GCG ATT GAG A
ACT1	ACT1-F	CCT ACG TTG GTG ATG AAG CT
	ACT1-R	GTC AGT CAA ATC TCT ACC GG

Results:

Genotyping analysis:

All tested isolates showing genotype A pattern *Candida albicans* with a PCR product size 450bp (figure 1), this compatible with the previous data indicate that the genotype A *Candida albicans* is the most common genotype in the clinical isolates.[16]

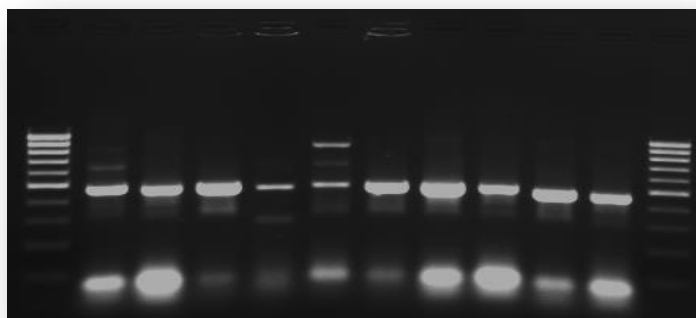


Figure 1: Agarose gel plate stained with Ethidium bromide showing a PCR amplified products of *C. albicans*; Lane (on border), DNA molecular size marker (100bp ladder); other in between show genotype A *Candida albicans* (450bp). (1.5% agarose gel, 100V-1hour).

SAP10 gene expression analysis:

The relative change of SAP 10 gene expression in different conditions was done by comparing the gene expression levels on the RPMI1640 medium as target gene to the gene expression on SAB medium as a

calibrator using (ACT1) gene as a reference gene. The results of this study showed that the level of SAP10 gene expression in RPMI1640 condition is different between different study strains.(Figure2)

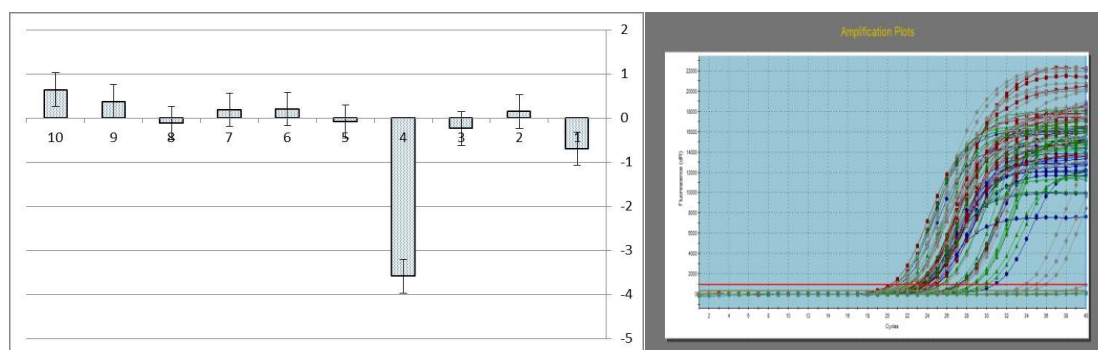


Figure2 : Real-time PCR expression profiling of SAP10 gene in RPMI1640 (as a target) and SAB condition (as a calibrator); the figure showing different gene expression in different strains.(The X-axis represents the strains and the Y-axis represents fold changes expression)

Some strains showed up-expression of SAP10 gene when grow in RPMI 1640 in comparison with *Candida albicans* grow on SAB medium ranging between (0.14 to 0.64 folds up) in strains 2,6,7,9 and 10,

while other strains showed down regulation ranging between (3.5 to 0.08 down folds) in strains 1,3,4,5 and 8.(Table 1) with no statistical significant pattern.

Table 1: Relative expression of SAP 10 gene in RPMI1640 medium in comparing to gene expression on SAB medium.

Fold expression Means \pm S.E.	Strain
-0.697 \pm 0.196 ^{ab}	S1
0.148 \pm 0.013 ^{ab}	S2
-0.240 \pm 0.184 ^{ab}	S3
-3.588 \pm 3.541 ^b	S4
-0.082 \pm 0.226 ^{ab}	S5
0.204 \pm 0.158 ^{ab}	S6
0.187 \pm 0.019 ^{ab}	S7
-0.115 \pm 0.009 ^{ab}	S8
0.375 \pm 0.177 ^{ab}	S9
0.641 \pm 0.019 ^a	S10

P<0.05

Discussion:**Genotyping:**

Genotype (A) *Candida albicans* considered as the most common genotype in *Candida* species isolated from human and animal clinical cases[16][17][18], the result of this study agree with this finding, the relation of this genotype with the virulence of *Candida albicans* is not clearly approved and no evidence indicate that the virulence genes located on the group I intron segment in the 25S rDNA (which is absent in the genotype A *Candida albicans*) [16] but still this procedure considered as a golden standard for confirm *Candida* isolation on species level and differentiate *Candida albicans* from *Candida dubliniensis* which both showing green colonies on Candida-CHROMagar and showing same biochemical reactions[12][19]

SAP10 Gene expression

The role of SAP genes family in the virulence of *Candida albicans* were tested previously [20][21], and the role of SAP10 gene in

pathogenicity process of *Candida albicans* was confirmed especially in the preserving of regulatory surface integrity of the yeast cells [1] while considered as a virulence key in some other studies[22] in addition to it is role in the presence and migration of some proteins (secreted proteins) [23]. In case of biofilm formation, no changes in gene expression levels was observed on contrary to the other SAP gene family [24] and at the same time there are an evidences indicate that the human blood effects on some SAP genes expression[25][26] but these studies did not focused on SAP10 gene in particular.

This study indicate that the SAP10 gene expression is vary in different study strains and there are no clear constant pattern for SAP10 expression related to growth condition and this agreed with Staniszewsk et al finding[27][28] when they test the effects of serum and other conditions on SAP10 gene expression.

Conclusion:

This study indicate that the SAP10 gene expression in *Candida albicans* is related to the *Candida* strains itself and no evidence indicate that the growth condition or 25s rDNA genotyping are related to SAP10 gene expression in *Candida albicans*.

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