Volume 8, Issue 5, 2018, 3590 - 3593 **ISSN 2069-5837**

Biointerface Research in Applied Chemistry

www.BiointerfaceResearch.com

Original Research Article **Open Access Journal**

Received: 10.09.2018 / Revised: 08.10.2018 / Accepted: 10.10.2018 / Published on-line: 15.10.2018

Genotypic characterization of adhesion and biofilm development genes in *Candida albicans* strains isolated from different clinical specimens

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ABSTRACT

Nowadays, *Candida albicans* is considered an important cause of invasive infections, especially in immunocompromised patients. The antifungal resistance and virulence properties of *Candida albicans* strains are a growing health problem worldwide. *Candida albicans* possess many virulence factors to lead to occurring lethal infections in patients with immunity deficiency. The aim of the present study was to investigate the adherence genes profiles of 16 *C. albicans* strains isolated from different clinical specimens. The *C. albicans* adhesion genes that most frequently isolated were: ALS3 (56.25% of the investigated isolates), ALS6 (50%), ALS4 (37.5), ALS1 (31.25), SAP7 (50%), and SAP8 (56.25%).

Keywords: *Candida albicans*, adherence genes, biofilm formation.

1. INTRODUCTION

Candida albicans is the most common yeast that causes fungal infections worldwide [1] being considered the major fungal pathogen causing lethal infections in immunocompromised patients. Various virulence factors are contributing to the colonization and pathogenicity of *Candida* sp. infection, including the adhesion and invasion on the cell surface, yeast-hyphae morphogenetic transformation, biofilm formation, phenotypic switching and the secretion of hydrolytic enzymes [2]. Nowadays, an expanding number of yeasts are becoming resistant to antifungal drugs and this is mainly attributed to biofilm formation [3]. Most of the hydrolytic enzymes virulence factors are extracellularly secreted by the fungus. The most well-known hydrolytic enzymes produced by *C. albicans* are secreted aspartic proteinases (Saps) which play a major role in overgrowth of the *Candida spp*. since these enzymes pave the way for adherence, penetration and tissue invasion [4]. Many studies suggest that the majority of infections produced by this pathogen are associated with biofilm growth [5]. The ALS gene family of *C. albicans* encodes large cell-surface glycoproteins that are involved in the process of adhesion to host surfaces [6]. The EAP1 gene was isolated as a presumed cell wall adhesion. Sequence analysis of EAP1 demonstrates that it includes a signal peptide, a glycosylphosphatidylinositol anchor site, and has homology to many other types of yeast genes encoding cell wall proteins. Additionally it is involved in the fungal adhesion to polystyrene [7].

2. EXPERIMENTAL SECTION

2.1. Clinical strains.

A total number of 16 nosocomial *C. albicans* were isolated from patients admitted for surgery in the Institute of Cardiovascular Diseases Prof. C.C. Iliescu, Bucharest, Romania, aged 20–85 years. The fungal strains were isolated from different anatomic sites [i.e. respiratory tract secretions (n=8), other secretions (n=6), and urinary tract infections (n=2) and diagnosed by the Vitek II automatic identification system.

2.2. Genotypic characterization of adherence genes of the tested strains.

The genetic support of the adherence genes (respectively the ALS 1 - 9, EAP1 and SAP 1 – 10 genes) was investigated by simplex and multiplex PCR [8], using a reaction mix of 20 or 25 µl (PCR Master Mix 2x, Thermo Scientific) containing 1 µl of *Candida* DNA extracted using the protocol of small scale isolation of DNA from yeast cells: the protocol include two main steps: i) preparation of the yeast cells by using buffers and zymolyase enzyme to eliminate the cell wall; ii) DNA extraction by using solutions A and B provided with the same kit. (Invitrogen, Life Technologies). All PCR reactions were performed using the Thermal Cycler machine Corbet. The amplification products were visualized by 1% agarose gel electrophoresis stained with the specific weight marker (100pb, Ladder Bench Top, Promega, USA. The amplification program, reaction components and primer sequences are shown in tables no.1, 2 and 3.

Table 1. Amplification conditions used in the PCR reactions.

Genes	Condition of amplification			
	denaturation	annealing	final extension	cycles
ALS2;ALS4;ALS5;ALS6;ALS7;ALS8;SAP6; EAP1	94° 5min	60° 30 sec	72° 30sec	35x
SAP5; SAP7; SAP9	94° 5min	57° 30 sec	72° 30sec	35x
SAP1; SAP2; SAP3	94° 5min	62° 30 sec	72° 30sec	35x
SAP4; SAP8; SAP10	94° 5min	55° 30 sec	72° 30sec	35x

Table 2. Reaction components used in the PCR reactions.

Concentration						Final volume
primer	$MgCl_2$	dNTP	DNA Taq-pol	Reaction buffer	DNA	
0,5µM	1,2mM	2µM	0,2U	1x	10x	20µl

Table 3. Primer sequence and amplicon primer size for the investigated adherence genes in *C. albicans.*

The gene	The primer	Primer size	Primer sequence
ALS1	**ALS1 F**	**183 bp**	F:CCTATCTGACTAAGACTGCACC
	ALS1 R		R:ACAGTTGGATTTGGCAGTGGA
ALS2	**ALS2 F**	**362 bp**	F:GCTGGCACCAACACAGTTAC
	ALS2 R		R:CGATAACCAGCGGGGACATT
ALS3	**ALS3F**	**167bp**	F:ACCTGACTAAAACTGCACCAA
	ALS3R		R:GCAGTGGAACTTGCACAACG
ALS4	**ALS4F**	**190 bp**	F:GTGCTGGTGACACATTCACG
	ALS4R		R:ATGGCTTTGGTGTCAGCAGT
ALS5	**ALS5F**	**826 bp**	F:TGCTGTGTTGGGTTGGTCAT
	ALS5R		R:ACCGTTAGATGCGGCATCAC
ALS6	**ALS6F**	**224 bp**	F:AGCTTGGACGGAACACTAGC
	ALS6R		R:GTGACGTACCAAACGCTCT
ALS7	**ALS7 F**	**880 bp**	F:CTATTGCCAGTCCCGGTGAT
	ALS7 R		R:TGGAGTCGGGAAATGAAGGG
ALS8	**ALS8 F**	**475 bp**	F:TTACAAACCCTGAGTCCGCC
	ALS8 R		R:TGGGGTTCCTGGTCCCTTAT
ALS9	**ALS9 F**	**1211 bp**	F:TGACTCATTGACATGGACTAGAT
	ALS9 R		R:GAATTTGCACAATAACAGTGTCTATG
SAP1	**SAP1 F**	**161 bp**	F:TCAATCAATTTACTCTTCCATTTCTAACA
	SAP1 R		R:CCAGTAGCATTAACAGGAGTTTTAATGACA
SAP2	**SAP2 F**	**81 bp**	F:TCCTGATGTTAATGTTGATTGTCAAG
	SAP2 R		R:TGACCATTAGTAACTGGGAATGCTTTAGGA
SAP3	**SAP3 F**	**231 bp**	F:CCTTCTCTAAAATTATGGATTGGAAC
	SAP3 R		R:TTGATTTCACCTTGGGGACCAGTAACATTT
SAP4	**SAP4 F**	**171 bp**	F: TTATTTTTAGATATTGAGCCCACAGAAA
	SAP4 R		R:GCCAGTGTCAACAATAACGCTAAGTT
SAP5	**SAP5 F**	**277 bp**	FF:AGAATTTCCCGTCGATGAGACTGG
	SAP5 R		R:TGACCATTAGTAACTGGGAATGCTTTAGGA
SAP6	**SAP6 F**	**187 bp**	F:CCCGTTTTGAAATTAAATATGCTGATGG
	SAP6 R		R:ACCAATACCAAGGGTATC
SAP7	**SAP7 F**	**196 bp**	F:TCTCAAGAAATTATCCCCCAAAATA
	SAP7 R		R:TCGGTTCCATTATCAGAATTTGTTC
SAP8	**SAP8 F**	**256 bp**	F:TCTCAAGAAATTATCCCCAAAATA
	SAP8 R		R:TCGGTTCCATTATCAGAATTTGTTC
SAP9	**SAP9 F**	**80 bp**	F:ATTACTCCACAGTTTATATCACTGAAGGT
	SAP9 R		R:CCACCAGAACCACCTCAGTT
SAP10	**SAP10 F**	**80 bp**	F:CCCGGTATCCAATAGAATCGA
	SAP10 R		R:TCAGTGAATGTGACGAATTTGAAGA
EAP1	**EAP1 F**	**704 bp**	F:GTTCCTAACAGGTCCACACCA
	EAP1 R		R:TCGCCACTTGCAGTAACAAC

3. RESULTS SECTION

The most frequently encountered adherence genes were ALS3, SAP8, ALS6 and SAP7, suggesting that they significantly contribute to the overall virulence of *C. albicans* strains isolated from symptomatic infections, presumably by facilitating the adherence to cellular and inert substrate. Several genes are involved in *Candida* sp. biofilm formation [9,10,11], among which, a major role is palyed by the agglutinin-like sequence (ALS) proteins, a family (ALS1-ALS9), the ALS genes which

encode glycosylphosphatidylinositol (GPI)-linked cell surface glycoproteins. Of all ALS proteins, the hypha associated adhesin ALS3 harbors an important role in adhesion [12, 13]. It was also demonstrated that the expression of several ALS genes is upregulated during biofilm formation; furthermore, the ALS proteins have long been considered excellent candidates for the investigation of fungal biofilm adhesion capacity [14]. The association between biofilm production and polymorphisms in the ALS3 central domain was analyzed by Bruder-Nascimento et al., and have shown to be not directly related to biofilm production development capacity [15].

The PCR results regarding the prevalence of ALS genes revealed the following decreasing order of the investigated genes: ALS3 (56.25%), ALS6 (50%), ALS4 (37.5%); ALS2 (12.5%) and only 6,25% of the nosocomial strains harbored the ALS8 gene (fig. 1 and table 4), opposite with other studies [16].

SAP (secreted aspartyl protease) genes are associated with a number of putative virulence attributes of *C. albicans* strains including hyphal formation, adhesion and phenotypic switching, highlighting the complexity of SAP involvement in *C. albicans* pathogenicity [17]. The proteolytic activity of the Sap proteins is involved in the degradation of the host barriers during infection [18], immune response evasion [19], and adhesion to the host cells [20].

In decreasing order of their abundance, the percentages of genes encoding for secreted aspartyl proteases were: SAP8 (56. 25%), SAP7 gene (50%) and SAP2 gene (6.25%), while other SAP genes (SAP1,SAP3,SAP4,SAP5,SAP6,SAP9 and SAP10) weren't identified in all tested strains. (fig. 1 and tab. 4), Monroy-Pérez et al., demonstrated higher percentages of SAP (94.8%) and ALS (35.8%) genes in clinical isolates of *C. albicans* collected from women visiting gynecologic services, while ALS1, SAP4–SAP6 were identified in all tested strains [21].

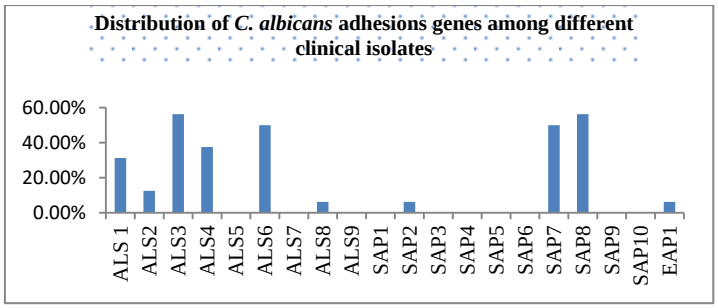

Figure 1. Genotypic characterization of adherence genes in *C. albicans* isolates.

Morphology-independent proteins (Eap1) can also contribute to adhesion, including GPI-linked proteins [22]. Our results showed that 6. 25% of the clinical isolates harbored EAP1gene (fig. 1 and tab. 4), a percentage similar to the one revealed by the study of Shalal et al., [16].

Table 4. The isolation sources and genotypic results in the investigated strains.

Strain code	Laboratory code	Isolation source	Adherence genes				
Candida albicans 58	1	Sputum	SAP7	SAP8	ALS8	ALS4	ALS3
Candida albicans 81	2	Sputum	SAP8				
Candida albicans 600	3	Tracheal secretion	ALS1				
Candida albicans 175	4	Sputum	ALS1	SAP8	SAP2	ALS3	SAP6
Candida albicans 438	5	Other secretion	SAP7	SAP8	ALS4	SAP6	
Candida albicans 1458	6	Thracheal secretion	ALS1	SAP7	SAP8	ALS3	SAP6
Candida albicans 128	7	Bronchial secretion	ALS4	ALS3	SAP6		
Candida albicans 1617	8	Other secretion	ALS1	ALS3	SAP6		
Candida albicans 904	9	Urine	SAP7	SAP8	ALS3	SAP6	
Candida albicans 527	10	Other secretion	ALS1	SAP7	ALS3	SAP6	
Candida albicans 324	11	urine	ALS3	EAP1			
Candida albicans 1726	12	Tracheal secretion	SAP7	SAP8	ALS4	ALS3	
Candida albicans 373	13	Bronchial secretion	SAP7	SAP8			
Candida albicans 381	14	Tracheal secretion					
Candida albicans 131A	15	Other secretion	SAP6				
Candida albicans 131B	16	Other secretion	SAP7	SAP8	ALS4		

4. CONCLUSIONS

In this study, the most frequent adhesion encoding genes in *C. albicans* were: ALS3 (56.25% of the investigated isolates), SAP8 (56.25%), SAP7 (50%), ALS6 (50%), ALS4 (37.5) and ALS1 (31.25). Suggesting that the products of these genes significantly contribute to the overall virulence of *C. albicans* strains isolated from symptomatic infections, presumably by facilitating the adherence to cellular and inert substrata. Further analysis is needed to study the sequence variation of these virulence genes in different geographically or clinically related isolates.

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6. ACKNOWLEDGEMENTS

The financial support of PN-III-P2-2.1-BG-2016-0369, granted by the Executive Unit for Financing Higher Education, Research, Development and Innovation (UEFISCDI) is gratefully acknowledged.