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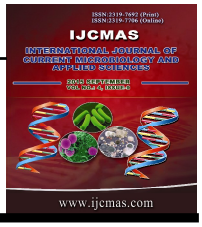


**Original Research Article**

# Detection of ALSt-1 biofilm gene from *Candida tropicalis* isolates

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ALSt-1 biofilm gene, *Candida tropicalis*, Immuno-compromised patients

**A B S T R A C T**

*Candida* is a genus of yeasts. Many species of this genus are endosymbionts of animal hosts including humans. Being a commensal, some *Candida* species have the potential to cause disease. Biofilm (BF) formation ability is an important virulence factor. Agglutinin like sequence (ALS) genes encodes adhesions in *Candida*. From the 200 immunocompromised patients 100 males and 100 were females. Among the 200, 51 were positive culturing on Sabouraud's agar medium. Among all the *Candida* isolates, 36 strains were *Candida albicans* 15 were non albicans. 17 isolates, biofilm positive by microtitre plate method. Among the 17 isolates 12 were non-*Candida albicans*. Among the 12,11 strains *Candida tropicalis*. Among the 11 isolates 9 strains were biofilm producers. After completing bio film formation, isolates processed for ALSt-1 biofilm gene detection.

## Introduction

*Candida tropicalis* is a species of yeast in the genus *Candida*. It is easily recognized as a common medical yeast pathogen, existing as part of the normal human flora. It was similar to *Candida albicans* in identification characteristics (Martin and White, 1981). The first example of a biofilm to be recognized in medical systems was dental plaque on tooth surfaces, but recent estimates suggest that a substantial proportion of human infections involve biofilms. In some parts of the world, *Candida tropicalis* has emerged as the second or the third most common agent of candidemia mainly in oncology patients (Kontoyiannis *et al.*, 2001; Leung *et al.*, 2002; Goldani & M´ario, 2003; Weinberger *et al.*, 2005; Vigouroux et al., 2006; Nucci & Colombo, 2007). In Latin America, particularly in Brazil, this species is also frequently isolated from blood of hospitalized nononcology patients (Godoy *et al.*, 2003; Goldani & M´ario, 2003; Colombo *et al.*, 2006; Nucci & Colombo, 2007). Moreover, the increased incidence of *Candida tropicalis* as a causative agent of

nosocomial urinary tract infections has been reported (Kauffman *et al.,* 2000; Alvarez-Lerma *et al.,* 2003; Rho *et al*., 2004; Jang *et al.,* 2005). Although *Candida tropicalis* is less prevalent than *Candida albicans*, it remains an important cause of human infections especially because of the high mortality rate of the patients (Costa *et al*., 2000; Leung *et al*., 2002; Goldani & M´ario, 2003; Bedini *et al*., 2006). In addition, the emergence of isolates less susceptible to azoles has been increasing (Hajjeh *et al*., 2004; Yang *et al*., 2004). ALS genes conform to a basic three-domain structure that includes a relatively conserved 5' domain of 1299 to 1308 nucleotides (433 to 436 amino acids), a central domain of variable length consisting entirely of a tandemly repeated 108-bp motif, and a 3' domain of variable length and sequence that encodes a serine-threonine-rich protein (Hoyer *et al.* 1998b).

Biofilm (BF) formation ability is an important virulence factor. Agglutinin like sequence (ALS) genes codes adhesins in Candida (Galan–ladero M A, 2013).

## Material and Methods

### Collection of Samples

Various clinical samples were collected from 200 patients visiting the Meenakshi medical college and hospital in Kanchipuram, Tamil nadu. Such as urine, sputum, throat swabs, vaginal swabs. During Jan 2012 to Jan 2013. All patients were immunocompromised.

### Biofilm formation

Sterile 96-well microplates were used to evaluate biofilm formation (26). By using a loop, a spot of each isolate was placed into tubes containing 2 mL of brain heart infusion broth(BHIB) medium with glucose (0.25%) and incubated at 37 °C for 24 h. Then all tubes were diluted at a ratio of 1:20 by using freshly prepared BHIB. From this final solution, 200 µL was placed into the microplates, which were then incubated at 37°C for 24 h. After incubation, the microplates were rinsed with PBS 3 times and then inverted to blot. Then 200 µL of 1% crystal violet was added to each well, followed by incubation for 15 min. After incubation, the microplate was again rinsed with PBS 3 times. Then 200 µL of ethanol :acetone mixture (80:20 w/v) was added to each well. They were read at 450 nm using an enzyme-linked immunosorbent assay (ELISA) reader (Biorad) and the OD was recorded for each well. Two wells were used for each strain. Staphylococcus aureus ATCC was employed as the control strain. Sterile BHIB without microorganism was employed as the negative control. The cutoff value was determined by arithmetically averaging the OD of the wells containing sterile BHIB and by adding +2 standard deviation. Samples with an OD higher than the cutoff value were considered positive, whereas those with lower value than cutoff were considered negative.

### Detection of alst1 gene from candida tropicalis by PCR

### Material & Methods

DNA purification kit (PureFast® Bacterial Genomic DNA purification kit), PCR Master Mix, Agarose gel electrophoresis consumables and Primers are from HELINI Biomolecules, Chennai, India.

### 2X Master Mix

It contains 2U of Taq DNA polymerase, 10X Taq reaction buffer, 2mM MgCl2, 1µl of 10mM dNTPs mix and PCR additives.

**Agarose gel electrophoresis**

Agarose, 50X TAE buffer, 6X gel loading buffer and Ethidium bromide are from HELINI Biomolecules, Chennai.

Pellet is suspended in 200µl of PBS. Added 50µl of Lysozyme and incubated at 37ºC for 15min.

400µl of Lysis buffer and 40µl of Proteinase K [10mg/ml] is added and gently mixed well. Incubated in water bath at 70°C for 10 min. Transferred whole lysate into Pure Fast spin column and centrifuged at 10000rpm for 1min. Discard flow through and added 500µl of Wash Buffer and Centrifuge at 10000rpm1 min. Discard flow through and added 500µl of Wash Buffer-2 and centrifuged at 10000rpm for 1min. Repeated wash one more time. Discarded flow through and Centrifuged column for additional 2 minute to remove any residual ethanol. Eluted DNA by adding 100µl of Elution Buffer and Centrifuged for 1min. Quality and Quantity of extracted DNA is checked by loading in 1% agarose gel and 1µl of extracted DNA is used for PCR amplification.

**Result and Discussion**

From the 200 immunocompromised patients 100 males and 100 were females analysed. Among the 200, 51 (25.5%) were positive culturing on Sabouraud's agar medium.

**Biofilm formation**

Among all the *Candida* isolates, 36 strains were *Candida albicans* 15 were non albicans. 17 (33.33%) isolates, biofilm positive by microtitre plate method. Among the 17 isolates 12 were non-*Candida albicans*. Among the 12,11 strains are *Candida tropicalis.*

**Results for detection of Alst-1 gene sequences in *Candida tropicalis* by PCR amplification**

Among the 12, 11 isolates were *Candida tropicalis* strains, the ALSt-1 gene was positive in 9 strains and negative in 2 strains. Self designed primer was used for detection of ALSt-1 gene in *Candida tropicalis* and this gene was sequenced and its confirmed by 16s rRNA (calibarator).

**Primer used for ALSt-1 Gene detection**

Forward Primer: 5'-
GTGACACAGAGTGTTCCTGATAA-3'
Reverse Primer: 5'-
TGGTAGTACATTGAGTGGTGTATG-3'
16S rRNA Gene: [Housekeeping gene]
Forward primer: 5'-
TACGGGAGGCAGCAGT-3'
Reverse primer: 5'-
TATTACCGCGGCTGCT-3'
16S rRNA gene expression as "Normalizer"
ALS1 gene expressed strains as "Unknowns"
Control ALS1 unexpressed strain treated as "Calibrator"

PCR Product size: 450bp

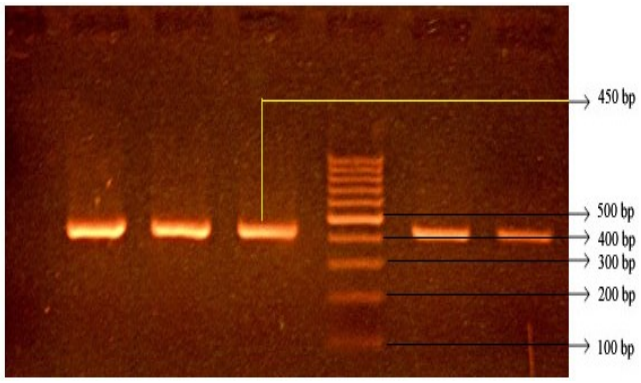
**Gene sequence**

**>AF201686.1| *Candida tropicalis* agglutinin ALST1 gene**

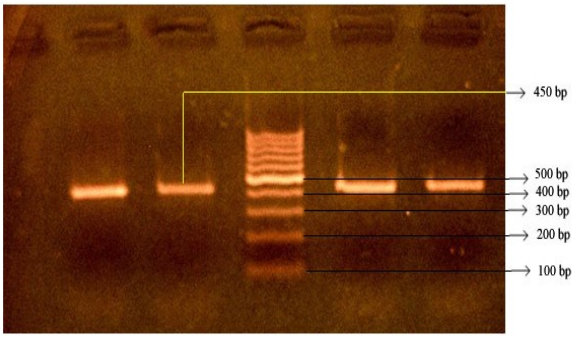
GGCATATCAATGAAAAGAGTTGCTGT
TTCTCCAGCAAATGTAAATGTATCAC
CGGCAGCCAATGCCGGTGACACATTT
ACCTTAATTATGCCATGTGTTTTTAAA
TTTACTACTAGTGAAACTTCTATTGAT
TTAACTGTGGGTAGTAAATCCTATGC
TACTTGTAATTTCAATGCTGGGGAAC
ATTTTACCACTTTTTCTAGTTTGAGTT
GTACTGTGACACAGAGTGTTCCTGAT
AATACCAATGCATATGGTACAATCAC

TGTTCCACTTGCCTTTAATGTTGGGGG
CTCTGGTCGTGATGTCGATCTTACTG
ATGCAAAGTGTTTTACTACAGGTGAT
AATACTGTTACATTTAGTGATGGTGA
TAAATCATTCTCCACTACAGCAAATT
TCGAAGGTGCCGGTACTTTGAATGAC
GATTATGAATCTTCAAGACTCATTCC
CTCACTTGGTAAAACTGATGCTTTGTT
GGTTGCACCATTGTGTTCTAATGGGT
ATAAATCAGGTACTATTGGGTTTTCC
TCAACAACAAAGGGTTTTTCAATTGA
TTGTAACAATATTCAAGCTGGTATTA
CTAGTCAATTGAATGCATGGGGTTTT
CCAACGGACCTGCAAAGCTTTTCATA
CACCACTCAATGTACTACCACTAGTT
ATTCCATAACTTTTAGTACTATTCCAA
AAGGTTTACGTCCATTCATTGATGCTT
ATATTAAAGCACCTACTTCCACATAC
CCCATGACATACACTTACAAATATGT
TTGTTCCGATGGAAAATCATATAATG
GCAATACAAAATTGAATTGGTCGGGA
TATGTTAACAGTGATGCAGATTCTGA
AGGTATGGAAATTGTTGTTGCTACTA
CTACTGGTACTGGTTCTACTACTGGT
GTTACAACATTACCATTTGATAAAAC
CAAAGACAAAACCAAAACAATTCAA
GTTATTGAACCAATTCCAACTACAAC
AGTTACTACTTCATACCTCGGTGTCA
CAACCTCTTTTTCGACCATTACTGCTA
CTATCGGAGAAACAGCTACTCTTGTC
ATTGATATGCC

Gel image of 9 *Candida tropicalis* ALSt-1 gene: All the 9 alst-1 genes was 450 bp size. Sample 1 to 5




Sample 6 to9




Among the 11 strains of *Candida tropicalis*, 9 strains produce biofilm formation in microtitre plate method. Among 36 strains of *Candida albicans* only 5 strains produces biofilm. During this study period *Candida tropicalis* shows high biofilm production than *Candida albicans*. So further study was done in *Candida tropicalis*, for the detection of biofilm ALSt-1 gene.

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