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Presence of an Intron Indicated in a *Candida krusei Tec1* Gene: A Preliminary Report

[1,2]Okungbowa Francisca I., [1]Pal Kalidas, [1]Chowdhury Rukhsana and [3]Okungbowa Michael O.A.

[1]Infectious Diseases Division, Indian Institute of Chemical Biology, Kolkata 700032, India
[2]Department of Plant Biology and Biotechnology (Formerly Botany), University of Benin, Benin City, Nigeria
[3]School of Medical Laboratory Sciences, University of Benin Teaching Hospital, Benin City, Nigeria

Corresponding Author: Okungbowa Francisca I., Department of Plant Biology and Biotechnology (Formerly Botany), Faculty of Life Sciences, University of Benin, P.M.B. 1154, Benin City, Nigeria

ABSTRACT

A *Candida krusei* clinical isolate was investigated for the presence of *Tec1* (a *C. albicans* virulence gene) using nucleotide amplification-based techniques. Amplification of genomic DNA did not show the presence of *Tec1* gene but the gene was expressed when Reverse-Transcriptase-Polymerase Chain Reaction (RT-PCR) was used. This is indicative of the presence of one or more introns in *Tec1* gene of the *C. krusei* which could play a role in chemotherapy. This is a preliminary report that requires further investigation.

Key words: Amplification, clinical specimen, DNA, gene expression, virulence

INTRODUCTION

There has been an increase in fungal infections in man over the past few decades (Sati and Joshi, 2011). The problem of increased fungal infection could be attributed to modern living conditions such as closed home environment, use of wall to wall carpets, poor water quality, among others (Bakheshwain *et al*., 2010). *Candida* species cause infection known as candidiasis. Candidiasis encompasses infections that range from superficial such as oral thrush and vaginitis, to systemic and potentially life-threatening diseases (Shekh *et al*., 2009). Cases of candidiasis have been on the increase over some decades (Chakravarthi *et al*., 2010; Chakravarthi and Haleagrahara, 2011). One of the previously uncommon *Candida* species, *Candida krusei* has become increasingly frequent in isolation (Melo *et al*., 2004; Ricardo *et al*., 2011). Cases of resistance to drugs in *Candida* infections have been ascribed to emergence of hitherto less frequent species, amongst them *C. krusei* (Sanglard and Odds, 2002; Pfaller *et al*., 2008). As a result, there have been increased interests in the understanding of the entire biology of *Candida* species. Most information on virulence in *Candida* is based on studies with *C. albicans*, hence information on virulence genes in other *Candida* species that are becoming common and problematic to treat when they cause diseases would be of interest.

The *Tec1* gene in *C. albicans* has been shown to regulate hyphal development (Dhillon *et al*., 2003; Argimon *et al*., 2007). *Candida albicans Tec1* is expressed in the hyphal form, and strains without *Tec1* are defective in the transcription of certain hyphal genes and show reduced virulence.

In the present study, a *C. krusei* strain isolated from a human male urethra was investigated for the presence of *Tec1*, as an initial step in determining its virulence status in comparison with *C. albicans*.

MATERIALS AND METHODS

***Candida* and *Saccharomyces cerevisae* strains:** This study was carried out between August 2004 and March 2005. The *C. krusei* strain was isolated from the urethra swab of a human male diagnosed of urinary tract infection at the Microbiology Laboratory of the University of Benin Teaching Hospital (UBTH), Benin City, Nigeria. The sample was grown on Yeast Peptone Dextrose, YPD Agar (0.5 yeast extract, 1 peptone, 2 glucose and 2% agar powder, BD France) with added chloramphenicol (50 μg mL^{-1}) at 37°C and maintained on YPD Agar slants at 4°C. Three clinical *C. albicans* strains (CA2, CA5 and CA10) isolated from women with vulvovaginal candidiasis in UBTH and *S. cerevisae* aα- diploid standard laboratory strain were included in the study as control strains.

Growth conditions: Culture plates of YPD Agar (in four replicates) were streaked separately with inoculum from a single colony of each *Candida* strain and incubated at 37°C, for 48 h, for colony and cell morphology studies. Wet preparations of cultures were examined with the x40 power of an optical microscope.

Strain identification: The *C. krusei* strain which was initially only identified as *Candida* sp. was re-identified by its colony and cell morphology according to Rao *et al.* (2004) and with the HiCandida identification kit (HiMedia, India), Chrom-Agar Candida according to Houang *et al.* (1997) and by comparison with a standard laboratory strain obtained from the Mycology Unit of the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria.

Detection of *Tec1*: Primers for *Tec1* were designed using primer 3 input software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) on the *Candida* genome sequence sites to get GCCATTTTGCTGTATCGTCA (forward) and CCGGGAAATTCAGAACAAGA (reverse).

The primers (Genuine Chemicals, India) were constituted in Tris- EDTA (10 mM Tris pH 8 plus 1 mM Ethylene-dimethyl trichloroacetic acid) buffer and stored at -20°C. They were then diluted to 25 mM with sterile distilled water.

Preparation of genomic DNA: Genomic DNA was isolated from overnight grown cells. The DNA was isolated from the *C. krusei* strain, one of the *C. albicans* strains (CA5) and the *S. cerevisae*, according to the method of Scherer and Stevens (1987). Briefly, tubes containing 5 mL of YPD broth were each inoculated with a loopful of stored cultures and incubated overnight at 37°C. A 1.5 mL aliquot of the overnight culture was centrifuged. Then 1 mL of 1 M sorbitol was added and centrifuged again. The packed cells were suspended in 1 mL of a solution containing 1 M sorbitol and 50 mM potassium phosphate buffer with 0.1% (v/v) 2-mercaptoethanol and 0.2 mg of zymolase (Miles Laboratories, Inc., Naperville) per mL. This was followed by incubation at 30°C for 30 min and centrifugation. The pellet cells were suspended in 0.5 mL of 50 mM sodium EDTA buffer (pH 8.5) with 2 mg of sodium dodecyl sulphate per ml to which was added 0.003 mL of diethyl pyrocarbonate, mixed and incubated at 70°C for 30 min. To this mixture was added 50 μL of 5 M potassium acetate, followed by mixing and incubation at 0°C for 30 min. Centrifugation was done

after which the supernatant was decanted into 1 mL of ethanol and mixed, then centrifuged and supernatant removed. Several volumes of 70% ethanol were added to the precipitate after which the ethanol was removed by pipetting and precipitate was allowed to dry. The precipitate was then suspended in 0.1 mL of 10 mM Tris HCl (pH 7.5) combined with 1 mM EDTA (TE solution) containing 10 μg of RNase A (Invitrogen) previously boiled for 1 min in TE solution at a concentration of 1 mg mL^{-1}). To the mixture were added 200 μL of 2-propyl alcohol followed by centrifugation and supernatant was decanted. An excess volume of ethanol was added to the precipitate and the ethanol removed as before. The precipitate was allowed to dry and then re-suspended in 0.05 mL of TE solution.

Amplification of DNA: The rRNA genes from the genomic DNA of the *C. krusei* strain, CA 5 (one of the *Candida albicans* control strains) and *S. cerevisae* were amplified as follows. The PCR mixture was made up of 50 ng genomic DNA, 1.5 mM $MgCl_2$, 200 μM dNTP, 0.5 μM primers, 2 U Taq DNA polymerase (Invitrogen) in 50 μL 1X-PCR buffer supplied by the manufacturer. Amplification was done for 35 cycles under the following conditions; 94°C/30 sec, 55°C/30 sec, 72°C/1 min and an extension period of 7 min at 72°C. Experiment was done twice and results compared for uniformity.

RT-PCR analysis: Ribosomal RNA was isolated from the three strains mentioned above, as follows. Strains were grown in YPD broth in triplicates, with shaking at 30°C for 18 h. The RNA was isolated and purified by the GITC (Guanidine isothiocyanate) -hot phenol method, as described by Collart and Oliviero (1993) and Ausubel *et al.* (1989) . The RNA was treated with RNase-free DNase -1 (1 U hg^{-1}, amplificaton grade, Invitrogen) in the presence of a RNase inhibitor (Rnasin, GIBCO-BRLO) and RT-PCR was performed with a single RT-PCR kit (INVITROGEN). The PCR products were electrophoresed on 2% agarose gels with ethidium bromide and gels were analysed using a Gel-DOC 1000 system (Bio Rad). PCR products were normalized according to the amount of rRNA detected in the same cDNA sample.

RESULTS AND DISCUSSION

Amplification of *Tec1* gene was not detected in *C. krusei* and *S. cerevisae* but in CA 2, CA 5 and CA 10, from chromosomal DNA of the strains (Fig. 1). However, RT-PCR analysis indicated that substantial rRNA synthesis occurred under the growth conditions. Also, *Tec1* gene was not expressed in CA 5 but in *C. krusei* (Fig. 2).

The *Tec1* gene is a member of a family of transcriptional factors which has the ability to regulate hyphal development and virulence in *C. albicans* and is expressed in the hyphal form, with strains defective in this gene showing reduced virulence as stated by Argimon *et al.* (2007). Understandably, *Tec1* was not detected in the *S. cerevisae* strain (which was a standard laboratory strain). The expression of *Tec1* in the *C. krusei* when RT-PCR was used suggests the presence of intron (s) in the strain. This agrees with the reports of Green *et al.* (2004) and Green *et al.* (2006), who said earlier that RT-PCR was an effective way of detecting *C. albicans* genes. The RT-PCR method has been used to confirm the differential expression of resistance phenotype in *C. albicans* reported by Xu *et al.* (2005). According to the report of Bhattacharya *et al.* (2000) in decoding the open reading frame of a gene for a known protein, one usually encounters periodic interrupting stretches of DNA (called introns), which get transcribed but not translated into protein. Since most genes are expressed as the proteins they encode, *Tec1* gene was not detected when chromosomal

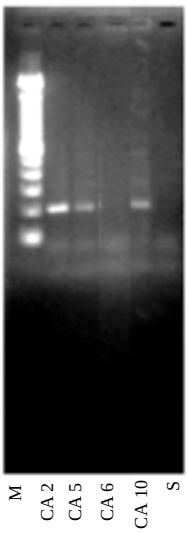

Fig. 1: Detection of *Tec 1* in *Candida* strains from chromosomal DNA; M = Marker, CA 2, CA 5, CA6 and CA10 = *Candida albicans* control strains; S = *S. cerevisae*

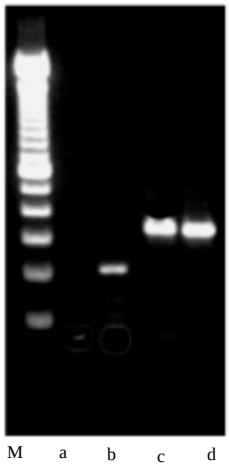

Fig. 2: RT-PCR analysis (M = Marker; a,b = CA 5 and *C. krusei*, respectively; c,d = ribosomal RNA of CA 5 and *C. krusei*, respectively)

DNA was used as a result of the likely presence of introns. This is in agreement with the explanation of Bhattacharya *et al.* (2000). Boucher *et al.* (1996) have used introns to study the relatedness among *Candida* species. A *Candida albicans* intron was found to interrupt the expression of a drug resistance gene and as such, the strain showed a high degree of drug susceptibility as reported by Mercure *et al.* (1997); this suggests a role in chemotherapy.

CONCLUSION

Introns play a role in antifungal chemotherapy. The presence of introns in this *C. krusei* strain needs confirmation and further investigation.

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