RAPD-PCR based genetic variation of *Candida albicans* of animal and human origin

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Abstract

The objective of this work was to compare the usefulness of a randomly amplified polymorphic DNA (RAPD) assay by using OPA-18 oligonucleotide for identification and genetic relation detection among different *Candida albicans* strains isolated from animals and humans. Thirty isolates were obtained from human and animals and genotyped in previous study according to 25S rDNA into genotypes A,B,C and T, identification was documented by germ tubes production, chlamydospores production on Corn-Meal-Tween 80 agar, characteristic green color on Candida-CHROMagar media (CHROMagar microbiology, France), and the final identification was confirmed by HiCandida Identification kit (HiMedia, India), RAPD-PCR fingerprinting which was carried out by using OPA-18 oligonucleotide. Results indicate that RAPD fingerprinting obtained by OPA-18 primer was not useful for *C. albicans* identification and these patterns were consistent under same genotype, but there were differences among different genotypes of *C. albicans*. Also this study indicates that same genotypic patterns were genetically identical but there was different degree of genetic relation between different genotypes. RAPD-PCR using OPA-18 primer was a very specific and sensitive method for epidemiological study at molecular level because of easy, reliable and highly sensitive to subgenotyping of *C. albicans* but it was of less value in identification of this microorganism.

Key words: *Candida albicans*, RAPD-PCR, OPA-18, fingerprinting, genotyping.

الاختلاف الجيني المبني على اساس تقنية تضخم الدنا العشوائي بتفاعل سلسلة البلمرة للمبيضات البيض الحيوانية والبشرية المنشأ

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الخلاصة

الهدف من هذه الدراسة هو مقارنة اهمية استخدام تقنية تضخم الدنا العشوائي بتفاعل سلسلة البلمرة باستخدام باديء OPA-18 لتحديد وايجاد العلاقة الجينية بين عزلات مختلفة من المبيضات البيضاء المعزولة من الانسان والحيوان. ثلاثون عزلة جمعت من الانسان والحيوان وصنفت جينيا بدراسة سابقة تبعا ل(25s rDNA) الى الصنف الجيني أ، ب، ج والصنف ت . التشخيص تم باستخدام Candida-CHROMagar ، تكوين الابواغ الجرثومية، تكوين الابواغ الزقية على وسط اكار دقيق الذرة المضاف له توين-80، واخيرا أكد التشخيص باستخدام HiCandida identification kit. اجريت البصمة الوراثية باستخدام تقنية تضخم الدنا العشوائي بتفاعل سلسلة البلمرة باستخدام الباديء OPA-18. اظهرت النتائج ان البصمة الوراثية المنتجة باستخدام باديء OPA-18 غير مفيدة لتشخيص عزلات المبيضات البيضاء ولكن الشكل الجيني الحاصل منها ثابت تحت الصنف الجيني الواحد المستحصل بطريقة (25s rDNA) ومختلف مع الاصناف الجينية المختلفة. وكذلك اثبتت هذه الدراسة ان الشكل الجيني الواحد الناتج باستخدام تقنية تضخم الدنا العشوائي بتفاعل سلسلة البلمرة هو متطابق جينيا ولكن هناك نسب متفاوتة من الاختلافات الجينية بين الاصناف الجينية المختلفة المستحصلة بطريقة (25s rDNA). ان تقنية تضخم الدنا العشوائي بتفاعل سلسلة البلمرة باستخدام باديء OPA-18 هي طريقة متخصصة جدا وحساسة للدراسات الوبائية المبنية على الاسس الجزيئية لسهولتها وحساسيتها العالية لتحت النوع الجيني للمبيضات البيضاء ولكنها غير مفيدة في تشخيص هذا الجرثوم.

الكلمات المفتاحية: المبيضات البيض، تضخم الدنا العشوائي، البصمة الوراثية، التصنيف الجيني.

Introduction

Dimorphic fungi from the genus *Candida* are present in humans and animals as harmless commensal or opportunistic microorganisms (1) causing serious and disseminated infections in cats, dogs, fowls, swine, cattle and horses or effect a single organ as is the case in piglet diarrhea and chicken thrush (2), urinary tract infections (3) or skin infection (4), while in humans causing wide range of locally and systemic infection specially in immunocompromized patients (5). Random amplified polymorphic DNA (RAPD) is used previously to characterize *Candida* isolates for medical and epidemiological studies (6, 7, 8). Despite the advantages of that RAPD profile provide a simple way for yeast identification at the species level and, in addition that RAPD could be useful in typing individual strains, routine identifications of yeasts by these genotypic methods have the important disadvantages of being laborious and time-consuming, as well as needing specialized equipment and, sometimes, requiring prior knowledge of nucleotide sequences in the target species (6, 9). In Iraq, there are previous studies about genotyping of *Candida albicans* on a genetic level according to 25S rDNA analysis in human and animals (10,11) and by RAPD technique in humans (12) but no previous studies about *C. albicans* isolated from animal origin and if it is related genetically with other strains from human origin. So, this study come to demonstrate the efficiency of PCR technique using OPA-18 primer as tool for *C. albicans* identification and focusing on the genetic relation of *C. albicans* isolated from animals and human origin by RAPD-PCR technique.

Materials and methods

A total of thirty *C. albicans* strains were obtained from clinical cases in humans (n=17) and animals (n=13) and genotyped previously according to 25S rDNA (10,11) as follows; genotype A: 11 isolates, genotype B: 10 isolates, genotype C: 5 isolates and genotype T: 4 isolates. In this study, the identification of all strains was confirmed by germ tubes production, chlamydospores production on Corn-Meal-Tween 80 agar, characteristic green color on Candida-CHROMagar media (CHROMagar microbiology, France), and the final identification was confirmed by HiCandida Identification kit (HiMedia, India).

DNA extraction: For DNA extraction, yeasts were grown on Sabouraud Dextrose Agar plates (Difco) at 37°C for 24h. A single colony was cultured overnight on YPD broth (1% yeast extract, 2% peptone and 2% dextrose) at 37°C and 200 RPM agitation. DNA was extracted using the DNA-Pure Yeast Genomic Kit (bio World, USA) according manufacturer's protocols. All DNA samples were stored at -20°C until use.

RAPD-PCR and Primer: Random amplified polymorphic DNA profiles were obtained with primer OPA-18 (5'-AGCTGACCGT-3') (BioCorp, Canada), according to a previously described method (9) with little modifications. The reaction mixture contained: 12.5 Robust 2G Hotstart Readymix (Kappa biosystem, South Africa), 2.5µl (25 pmol) of primer, 5µL DNA template and complete the volume by molecular grade water to 25µL final volume. The reaction mixtures were subjected to the following thermal cycling parameters in a TECHNE TC-300 thermocycler (Bibby Scientific, UK): 94°C for 3 min followed by 30 cycles of 94°C for 30 sec., 60°C for 15 sec., 72°C for 1 min and final extension at 72°C for 10 min following the last cycle. All reaction products were characterized by electrophoresis on 1.5% agarose-ethidium bromide gel in 1X TBE buffer at 75V for 75min. data analyzed by gel documentation system (SCIE-PLAS, UK), and gene tool analysis software (Syngene, UK).

Results

The results of the RAPD-PCR technique for *C. albicans* strains isolated from different sources by OPA-18 primer showed presence of four different patterns (Fig. 1), these fingerprints related to genotype patterns based on 25S rDNA and there was no difference between animal and human source isolates (Table 1).

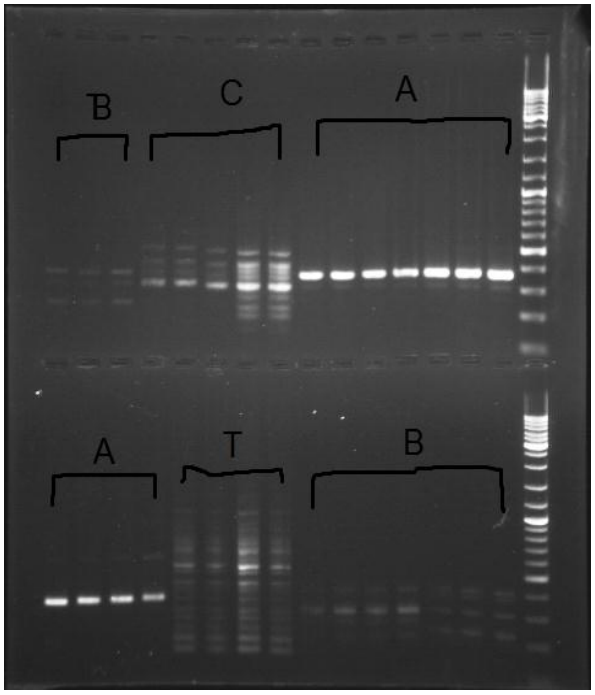

Fig. 1: RAPD-PCR fingerprinting using OPA-18 primer,*Candida albicans* genotype A, B, C and T.

Table (1): Molecular weights of the monomorphic RAPD bands considered for different genotypic isolates of *C. albicans*.

C. albicans* Genotype	**Number of strains**	**RAPD-PCR fingerprinting (bp)****
Genotype A	11	375
Genotype B	10	430, 330, 250
Genotype C	5	500, 440, 335
Genotype T	4	700, 570, 470, 390, 340, 300, 235, 200

***According to 25S rDNA, **Data analyzed by gene tool analysis software (Syngene, UK).**

Discussion

Molecular techniques based on RAPD-PCR have been used as a tool for diagnosis of several fungal species (13). The present study shows that fingerprints are specific for *C. albicans* subgenotypes but it's not useful for *C. albicans* detection. This conclusion conflicted by the finding of (14) and (15), who reported that RAPD-PCR patterns enabled the direct identification of *Candida* species, including *C. albicans* and contrary to (12) who found different RAPD patterns by using OPA-18 in her study to *C. albicans* isolated from vaginitis cases in Iraq, while data partially agree with (16) and (17), who found different patterns related to OPA-18 primer and they consider these patterns as identification feature. On the other hand, RAPD-PCR technique with OPA-18 primer showed specific, constant and different band patterns for each strains group. This is contrary to the results reported by (18), who mentioned that the RAPD-PCR patterns obtained with OPE-18 primer for identification of clinical isolates were consistent. The failure to obtain specific patterns for all *C. albicans* strains in this study raises the question of reliability of this primer in the diagnosis of *C. albicans*. This report revealed that using of RAPD with the primer OPA-18 is highly discriminatory for subgenotyping of *C. albicans* isolate from different sources. Consistent RAPD patterns were obtained by using OPA-18 oligonucleotide allowed rapid, accurate, reliable, and simple differentiation of *C. albicans* genotypes and this strongly supported by (14) and (19). In addition, using of RAPD fingerprints can aid in the study of nosocomial fungal-infection epidemiology by having the ability to delineate discriminate among strains of the various *Candida* species evaluated at the genetic level (19). This report indicate that RAPD fingerprinting obtained by OPA-18 primer was not useful for *C. albicans* identification and there are no genetic differences between *C. albicans* isolated from animal and human origin, as well it is useful in demonstration some correlations among *C. albicans* isolates under subgenotype level but it's a weak marker for epidemiological studies.

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