A Study Molecular of Some Genetic Virulence Factors for Yeast C. Albicans to Children in Ramadi City, Iraq

Dhuha Kamal Jasim Al-Ani[1], Harith K. Buniya[2], Farkad Hawas Musa[3]
[1, 2, 3] Department of Biology - College of Education for Pure Sciences/ - University of Anbar-IRAQ
E-mail; duhalove999@gmail.com

Abstract
This study aimed to diagnose molecular to some virulence factors for C. albicans isolated from oral premature Infants whose ages ranged from one day to one year in Ramadi city, Iraq. The morphological characteristics were diagnosed on SDA media, corn flour media, and differential CHROM medium for 90 samples, showing 39 samples of C. albicans small, light green colonies. The molecular study focused on studying the genetic factors responsible for some virulence factors, where the chromosomal DNA of four selected isolates was extracted from the study isolates, depending on what we obtained from the results of the virulence factors test by choosing two isolates with high virulence factors and two with weaker virulence factors. The purity and concentration of the extracted DNA were measured and used as a template to amplify (whp1), (sap1), and (als1) genes using specific primers, and DNA pieces were obtained, with sizes 896, 750 and 745 bp, respectively. The sequences of the nitrogenous bases of (sap1) and (als1) genes were identified and matched with the database in the NCBI and the results of the comparison showed variation in the sequences between the studied strains.
Keywords: PCR, c. albicans, Candidiasis, Oral Thrush

1. Introduction

Fungal diseases are usually caused by tiny living organisms called fungi, which can invade different parts of the human body, such as the lungs and skin, as well as other parts of the body such as the mouth, genital areas, and others. Fungi are usually found in the air, water, and soil, but there are some types of them, and not all of them cause human diseases. Although most people are not affected by fungi, even if they are affected by them, this effect is temporary, as the human body can resist and overcome them, especially people with high immunity. Some fungal pathogens are free-living, and they cause disease in humans through inhalation or their spores entering the body through cuts or scratches, and some of them are considered part of the normal flora of the human body, such as yeast and candida, such as Candida and others, and they are harmless and do not harm them unless the body has a weak immune system (Agustinho et al., 2018).
Fungal diseases have started to become more common recently, and they appear with different symptoms. The difficulty of infection lies in the fact that it grows and multiplies slowly, and its diagnosis is not an easy task. Most of them do not respond to treatment, but they rarely lead to death, except in the case of people with immunodeficiency and chronic diseases such as AIDS and HIV (Boral et al., 2018).
The C. albicans is one of the most widespread fungi and is one of the most important opportunistic pathogens. It remains present in the mouth for many years without showing any pathological symptoms, but Candida albicans may change from a commensal (non-pathological) state to a pathological state (Supriya et al., 2016), and examined yeast C. albicans by microscope show large ovoid or spherical cells with a budding blast conidium, and the yeast can show as hyphae or pseudo hyphae growing at temperatures ranging from (20-38 C˚) (Zhu et al., 2015).
The genetic material of fungi constitutes four gene groups that enhance these fungi' ability to cause disease، C. albicans possesses a group of virulence genes that directly affect the mucous cavities of the mouth and vagina, including genes that contribute to the adhesion process, such as ALS, HWP, and SAP, The main steps for antifungal drug targets are by understanding the molecular mechanisms of pathogenesis and to reveal the antifungal targets, in addition to the properties of the natural or synthetic antifungal product with the hope of making progress and new insights to accelerate the improvement of new strategies for the treatment of dermatophyte diseases (Fahim et al., 2022). Therefore, the study aims to diagnose molecular to some virulence factors for C. albicans isolated from oral premature Infants in Ramadi city, Iraq.

2. Materials and Methods

Molecular diagnosis of *C. albicans*

DNA extraction

DNA was extracted from C. albicans of 4 isolates, using a Genomic DNA extraction kit, equipped with (Geneaid™ DNA Isolation Kit, Geneaid Biotech Ltd, Taiwan), and the extraction was carried out according to the company's instructions.

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Polymerase chain reaction (PCR) and preparing the primers

The PCR technique was used to diagnose the yeast Candida albicans by using primers with specialized sequences present within the fungal genome and designed in this study using NCBI-Gene Bank according to (Table 1). The working stock was prepared by adding deionized dd H2O to the required concentration according to the supplier's recommendation. The PCR mix was prepared using the AccuPower® Gold Multiplex PCR PreMix Kit prepared by the Korean company Bioneer. According to the company's instructions, the mixture was prepared in 20µl.

Table (1) The DNA primers C. albicans of virulence factors.

Size (bp)	Primer sequence → 5` 3`	Gene	
896	5- TCCAAATACATCTGTTCCAACCA-3 5-CAGTCGTAGAGACGACAGCA-3	F R	whp1
750	5- CTGGATCATCTGATTTATGGG-3 5-AACGGAGCAGTAAACTCA-3	F R	sap 1
745	5-TCAGATGCTTCAACAATTTACA -3 5-AGATGAAACCGGATAATTCCA-3	F R	als 1

The PCR program involved initial denaturation at 95°C for 5 min, 35 cycles (denaturation at 94°C for 45 sec, annealing at (54°C, 57,5°C and 60°C for 30-sec respectively to genes of als1, whp1, sap1) and extension at 72°C for 1 min) and final extension at 72°C for 7 min. The PCR products were run on 1% agarose gel containing 1 µl ethidium bromide using a 100 bp ladder (Bioneer) as a molecular weight marker. The gel was examined on an ultraviolet transilluminator and photographed.

Genes sequencing

The identification of als, sap and whp genes was carried out for four selected samples from the studied fungal isolates (two with high resistance and two with a low resistance to antibiotics). The PCR product was sent to the Korean company Macrogene to determine the DNA sequences using the Genetic analyzer device to obtain the nucleotide sequence of the gene in order to compare it with the sequences stored in NCBI.

3. Results and discussion

Chromosomal DNA extraction for c. albicans

The DNA was extracted from (4) isolates selected in this study according to biochemical tests and their ability to form virulence factors for final diagnosis using primers. Electrophoresis on agarose gel at a concentration of (1%) showed the appearance of one bundle representing the chromosomal DNA of the different subspecies (Fig. 1) was detected using ultraviolet light and ethidium bromide dye.

Figure (1): Electrophoresis of chromosomal DNA extracted from C. albicans isolates on agarose gel (1%) at a voltage of 70 mV for one hour.

Amplification gene

In this study on a group of virulence genes that directly affect the mucous cavities of the mouth and vagina and that contribute to the adhesion process, which are als1, hwp1 and sap1, using the polymerase chain reaction (PCR) technique and under the conditions that were previously mentioned in the work methods, the results showed that we obtained The required piece of DNA was obtained from one isolate, which is (D) only for the hwp1 gene, with a size of 896 bp, as shown in Figure (2), while the required piece of DNA was obtained from two isolates, which are (D, F) for the sap1 gene, with a size of 750 bp and three Isolates for the als1 gene have a size of 745 bp, as shown in (Fig. 3).

Molecular diagnosis by using PCR technology is one of the most accurate techniques used in the diagnosis process and determining related virulence factors, as it requires a "short" time and high-accuracy results that play an "important" role in identifying pathogenic microorganisms (Mahmood et al 2019; Salman et al 2020). This technique relies on reading the codes for a specific region on a tape DNA After extracting sufficient amounts of DNA, the specific primers are amplified and then the readable sequences are detected by electrophoresis on an agarose gel (Zhang et al., 2020).

Cavalcanti et al. (2015) they Studied indicated that there are many genes that are closely related to the formation of biofilms and make the yeast more resistant to the surrounding conditions of C. albicans, such as Hyphal Wall Protein (hwp1), Secreted Aspartyl Protease (asp1), Agglutinin-Like (als1) Sequence for its relationship to the encoding of cell wall proteins and the production of enzymes that contribute to the process of adhesion of C. albicans to the surface of the host.

Cota and Hoyer, (2015) found that the C. albicans isolates from the mouth possess the ALS1 gene, which encodes the production of Agglutinin-like protein 1, which is responsible for the process of adhesion of Candida albicans yeast to the cells and tissues of the host, which is the first step of the colonization process and occurs by the formation of a fibrous layer. It is composed of multiple sugars and has a glycoprotein nature that helps the yeast attach to proteins, carbohydrates, and host cell membranes.

The *hwp*1 gene (Hyphal Wall Protein 1) is a glycosylphosphatidylinositol-binding protein that encodes cell wall proteins of hyphae in the mature biofilm and regulates adhesion during biofilm development of yeast hyphae and is a substrate for host-

derived transglutaminase activity, thus it mediates binding *C. albicans* covalently attaches to host cells **(Semlali et al., 2014),** while the SAP gene is more virulent during polymicrobial infections. Genes belonging to the SAP family (SAP1-10) contribute to adhesion and infection through damage to host cell membrane components **(Cavalcanti et al., 2016)**. SAP5 is known as a protein that mediates adhesion. Fungal colonization of human oral tissues **(Ardehali et al., 2019)**.

Figure (2) Electrophoresis of agarose gel at 1% for PCR products for the whp1 gene.

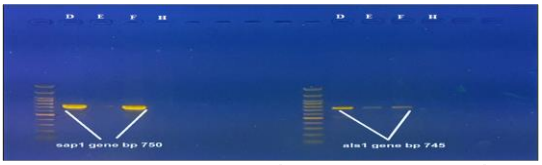

Figure (3) Electrophoresis of agarose gel at 1% for PCR products for the sap1 and als1 gene

Genes Sequencing

After the studying whp1, sap, and als1 genes in the samples of yeast C. albicans, the products of the polymerase chain reaction were determined by electrophoresis and compared to volumetric evidence, and the sequences of the nitrogenous bases of the samples were determined by the Sanger and Coulson method or by the chain termination method, as they were The samples belonging to the als1 and asp1 gene were sent, and the sequences were matched with the Basic Local Alignment Search Tool (BLAST). The program compares the sequence databases stored in NCBI and determines the validity of the results obtained through the percentage of similarity with the stored data. The matching results showed that the als1 gene was 99.15%, while the sap1 gene match rate was 80.59%. Then the nucleotide sequences of the als1 and sap1 genes were compared with each other, where the alignment was done using the MEGAX program for samples isolated from high-resistant strains with those that showed weak resistance. For antigens, the homology ratio between highly resistant and weak samples was 91.4% for the asp1 gene, and most variations were in the last 100 nucleotides of the studied segment. The results of the comparison between the als1 gene sequences showed that the homology ratio between them was 84%, as there was a discrepancy in the sequence of the nitrogenous bases between the high and weak strains, and the discrepancy was distributed along the variance was distributed along the sequence studied.

4. Conclusion

The results of electrophoresis of the extracted DNA showed one band, and the purity and concentration of the extracted DNA were measured and used as a template to amplify (whp1), (sap1), and (als1) genes using specialized primers, and DNA pieces were obtained, with sizes (896 bp), (750 bp)) (745bp), respectively.The sequences of the nitrogenous bases of the (sap1) and (als1) genes were determined and matched with the sequences of the same gene in the NCBI database and showed a similarity of 91.4% for the gene (sap1) and 84% for the gene (als1). The sequences of the studied strains were compared using the MEGA-X program. The results of the comparison showed a variation in the sequences between the studied strains.

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Appendix (2) gene sequences of als1on NCBI