



Molecular analysis of certain adhesion genes in *Candida albicans* isolated from chickens with thrush

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Abstract

Candida albicans causes inflammation in the oral cavity due to its possession of certain virulence genes that enable it to invade the epithelial cells of the mouth. The aims of this study were to molecularly identify *C. albicans* and to detect specific adhesion genes such as ALS1 and HWP1. Eighteen isolates of *C. albicans*, previously isolated from chickens with oral candidiasis in a study conducted at the College of Veterinary Medicine, University of Mosul, were examined in this research. Molecular diagnosis was performed using polymerase chain reaction (PCR), targeting the 18S rRNA, ALS1, and HWP1 genes to detect *C. albicans* and its virulence factors. The results showed that all *C. albicans* isolates produced PCR amplicon products at a molecular weight of 415 bp for the 18S rRNA gene. Additionally, 66% of the isolates yielded PCR amplicon products at a molecular weight of 577 bp for the ALS1 gene. However, all isolates tested negative for the HWP1 gene. Four isolates of *C. albicans* have been registered in GenBank with the following accession numbers: PO328951, PQ328952, PQ328953, and PQ328954. The isolates exhibited 97-100% similarity with global *C. albicans* isolates from China, the USA, and India. The study concluded that the isolates under investigation possess a high percentage of the ALS1 gene, which significantly contributes to their pathogenicity in poultry.

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Introduction

Oral Candidiasis (thrush) in chickens is one of the ecumenical infections that effects on poultry industry. Chickens, turkeys, geese, and parakeets are considered highly susceptible hosts to infection with this form of candidiasis (1). *Candida albicans* is a part of the flora in the oral cavity of the chicken, that is commensals on the mucocutaneous cells in the gastrointestinal tract of chicken (2,3) it is an opportunistic pathogen and causes thrush when the predisposing factors to infection are available as immunosuppressive conditions e.g. debilitation, malnutrition, overcrowding, poor hygiene, young chicken less than 4 weeks and concurrent disease (4). Also, when using the long-term therapy of antimicrobial drugs (5). The main sources for candidiasis are contaminated food or drinking water with *C. albicans* from the environments e.g.

human waste, and droppings of birds (6,7). *C. albicans* causes types of inflammation in the mouth, esophageal, gizzard, and crop due to its possessing virulence genes, which permit them to invade the host tissue and avoidance of defense mechanisms (8) as extracellular enzymes, including phospholipases, aspartyl proteases that contribute in adhesion to the epithelium cell, degrade proteins of the extracellular matrix (9,10). In addition, virulence factors of *C. albicans* are very linked with the nature of pathogens as adherence and coaggregation, phenotype switching, interference with the immune system as immunomodulation, and antibiotic resistance (11,12). In the ALS (Agglutinin-Like Sequence) gene family, the agglutinin-like sequence is an important family related to adhesions in *C. albicans* (13,14). The members of this family interact with several substrates, including the epithelial cells of the host besides proteins (15,16). Hyphal wall protein 1 (*HWP1*) another

adhesion gene, is regulated at the time of the development of biofilm. HWP1, glycosylphosphatidylinositol (GPI) linked mannoprotein. It is a substrate for transglutaminase activity acquired from a host; thus, it moderates the covalent affinity of *C. albicans* with the host cells (17). *C. albicans* is a Gram-positive microorganism, a polymorphic yeast that reproduces by budding and chlamydo spores and forms pseudohyphae (18,19). These features depend on the diagnosis, besides the formation of a germ tube in serum through 2h at 37°C (20). Molecular diagnosis is important for confirming isolates and detecting the genes that contribute to their virulence (21).

Previous genetic studies of *C. albicans* have detected that virulence factors might be correlated with the genotypic profiles. The molecular diagnosis that is more sensitive and specific therefore has been used for these analyses (10), therefore, this study aimed to detect *C. albicans* molecularly and their Adhesion genes as *ALS1*, and *HWP1*.

Materials and methods

Ethical statement

The Institutional Animal Care and Use Committee (UM.VET.2024.104) 18/8/2024 licensed this study.

Isolates

Eighteen isolates of *C. albicans*, previously isolated from chickens with oral candidiasis in a study conducted at the College of Veterinary Medicine, University of Mosul, were

examined in this research. Methods used for isolation and diagnosis of *C. albicans* were mentioned in the study (1). The isolates were enhanced on Brain Heart Infusion agar at 37°C for 18h. and subculture on Sabouraud Dextrose agar at 37°C for 48 h. Then, isolates were confirmed by culturing them on Candida Chromogenic agar (CONDA com, Spain).

Germ tube formation

A germ tube formation test was used to distinguish *C. albicans* from other species, by injecting a one colony of suspected isolates in 0.5 ml of human serum and incubating at 37°C /3 h. (22).

DNA Extraction

All *C. albicans* isolates were subjected to DNA extraction for molecular conformation and detection of *ALS1* and *HWP1* genes, the extraction of DNA was done by using Add Prep Genomic DNA Extraction (Korea) according to the company's instructions, and the DNA concentration was determined using Nanodrop (Nano Photometer® N50/ Germany). The DNA stored in -80 °C until used. The 25µl of PCR mixture (1 µl from each primer, 10 µl of GoTaq® G2 Green Master Mix (Promega, USA), 8 µl of DNase-free water, and 5µl of extracted DNA) was used for amplification 18S rRNA gene specific for the detection of *C. albicans*, *ALS1*, and *HWP1*. All primer sequences and amplification cycles (Tables 1 and 2), and amplification was done by using conventional PCR (Sensoquest, Germany) (23,24).

Table 1: Amplification program for genes under study

| Type of PCR | Initial denaturation | Cycle numbers 35 | | | Final extension |
|-------------|----------------------|------------------|-----------|-----------|-----------------|
| | | Denaturation | Annealing | Extension | |
| 18S rRNA | 95/2 | 94/0.30 | 59 /0.30 | 72/1 | 72/5 |
| ALS1 | 95/2 | 94/0.30 | 58 /0.30 | 72/1 | 72/5 |
| HWP1 | 95/2 | 94/0.30 | 59 /0.30 | 72/1 | 72/5 |

Data expressed as °C/minutes.

Table 2: The primer sequences for genes under the study

| Gene primer | Sequence (5' – 3') | Products size | References |
|-----------------|--|---------------|------------|
| <i>18S rRNA</i> | F-GCCGCCAGAGGTCTAAACTT R- AGTTCAGCGGGTAGTCCTAC | 415 bp | (25) |
| <i>ALS1</i> | F-ACATGTACTGTTGAACGACGCT R-GACGACTGCCAGCACAAGTA | 577 bp | (26) |
| <i>HWP1</i> | F-TCTACTGCTCCAGCCACTGA R-TTCAGTGGCAGGACTGATG | 501 bp | (27) |

Sequencing of *Candida albicans* isolates

After the PCR amplification, the amplicons were sequenced (Macrogen, Korea). Using BLAST, the obtained gene sequences were compared with Partial DNA sequences of the agglutinin-like protein (*ALS1*) gene sequences from

other countries that had already been recorded in the GenBank. The phylogenetic tree of *C. albicans* isolated from local chickens in Iraq was organized to utilize the Maximum Likelihood technique based on the Tamura-Nei model in MEGA11 software (28,29).

Results

Germ tube formation:

According to the formation of the germ tube, the results revealed that all isolates formed the germ tube after 2-3 h on incubation on human serum at 37°C (Figure 1). On Candida Chromogenic agar, all *C. albicans* isolates showed green colonies (Figure 2).



Figure 1: Producing germ tube from *C. albicans*.



Figure 2: *C. albicans* produces green colonies on Candida chromogenic agar.

Molecular detection of *Candida albicans* and their virulence genes

The molecular identification of *18S rRNA* for *C. albicans* gave an amplicon product of 416 bp (Figure 3). The amplification of *ALS1* virulence genes resulted in 577 bp in 66% of *C. albicans* isolates (Figure 4). In contrast, the amplification of the *HWP1* gene yielded negative results for all tested *C. albicans* isolates.

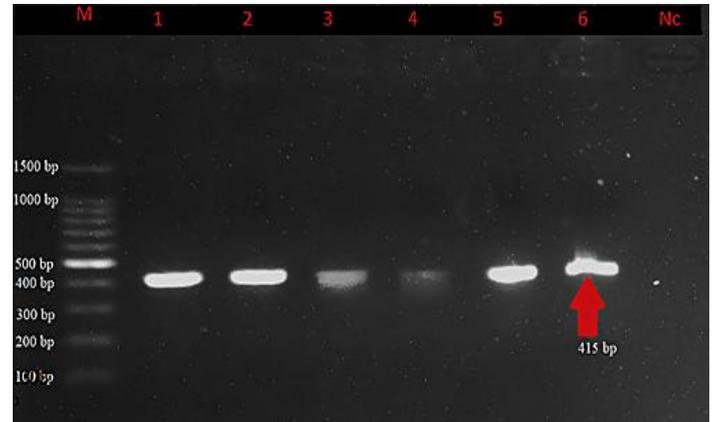


Figure 3: Amplification of the *18S rRNA* gene for *C. albicans* M= 100 bp. marker, 1-6 =positive isolates, Nc= negative control.

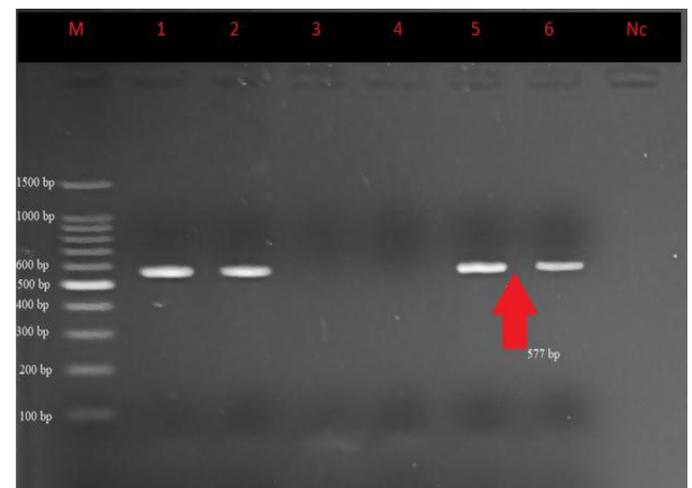


Figure 4: Amplification of the *ALS1* gene for *C. albicans* M= 100 bp. marker. (1-6) positive isolates, Nc= negative control.

Sequencing and phylogenetic tree

The genetic sequence of the isolates was determined, then the query alignment of *C. albicans* with the NCBI GenBank was determined, and its percentage of identity with global isolates was determined (Figure 5). Four isolates of *Candida albicans* were registered in the NCBI with serial numbers PO328951, PQ328952, PQ328953, and PQ328954. The isolates showed a 97-100% affinity with global isolates in China, USA, and India (Table 3). The phylogenetic tree of *C. albicans* isolated in Iraq and the degree of relatedness with global isolates were determined (Figure 6).

Sequences producing significant alignments

Download Select columns Show 100

37 sequences selected

| Description | Scientific Name | Max Score | Total Score | Query Cover | E value | Per. Ident | Acc. Len | Accession |
|---|------------------|-----------|-------------|-------------|---------|------------|----------|-------------|
| Candida albicans strain P2G.applutum-like protein (als1) gene, complete cds | Candida albicans | 1016 | 1016 | 100% | 0.0 | 100.00% | 980 | JQ034421.1 |
| Candida albicans strain P2S.applutum-like protein (als1) gene, partial cds | Candida albicans | 1016 | 1016 | 100% | 0.0 | 100.00% | 980 | JQ307472.1 |
| Candida albicans strain P2B.applutum-like protein (als1) gene, partial cds | Candida albicans | 1016 | 1016 | 100% | 0.0 | 100.00% | 980 | JQ307471.1 |
| Candida albicans SC5314.Als1z (ALS1) partial mRNA | Candida albicans | 1016 | 1016 | 100% | 0.0 | 100.00% | 3793 | XM_712984.2 |
| Candida albicans.applutum-like protein (ALS1) gene, ALS1-1 allele, complete cds | Candida albicans | 1016 | 1016 | 100% | 0.0 | 100.00% | 6971 | OR664373.1 |
| Candida albicans.applutum-like protein (ALS1) gene, ALS1-2 allele, complete cds | Candida albicans | 1016 | 1016 | 100% | 0.0 | 100.00% | 5793 | OR664374.1 |
| Candida albicans strain GP11.applutum-like protein (als1) gene, partial cds | Candida albicans | 1016 | 1016 | 100% | 0.0 | 100.00% | 980 | JQ307470.1 |
| Candida albicans.applutum-like protein (als1) gene, partial sequence | Candida albicans | 1011 | 1011 | 100% | 0.0 | 99.82% | 3573 | AY445055.1 |
| Candida albicans strain Y2X.applutum-like protein (als1) gene, partial cds | Candida albicans | 1011 | 1011 | 100% | 0.0 | 99.82% | 980 | JQ307473.1 |
| Candida albicans.applutum-like sequence (ALS1) gene, complete cds | Candida albicans | 1011 | 1011 | 100% | 0.0 | 99.82% | 3798 | L25902.1 |
| Candida albicans strain P2X.applutum-like protein (als1) gene, partial cds | Candida albicans | 994 | 994 | 100% | 0.0 | 99.27% | 980 | JQ307478.1 |
| Candida albicans.applutum-like protein (ALS1) gene, ALS-1 allele, complete cds | Candida albicans | 584 | 584 | 97% | 4e-162 | 86.43% | 3813 | AF068866.1 |
| Candida albicans strain Fungz-01 chromosome 6 | Candida albicans | 562 | 1419 | 94% | 2e-155 | 100.00% | 1154996 | CP115803.1 |
| Candida albicans strain P2S.applutum-like protein (als1) gene, partial cds | Candida albicans | 556 | 556 | 97% | 9e-154 | 85.50% | 980 | JQ307474.1 |
| Candida albicans.applutum-like protein (ALS1) gene, complete cds | Candida albicans | 556 | 556 | 97% | 9e-154 | 85.50% | 4723 | AF025429.1 |
| Candida albicans cell-surface adhesin (ALS1) gene, ALS-small allele, complete cds | Candida albicans | 551 | 551 | 97% | 4e-152 | 85.32% | 4044 | AY227439.1 |
| Candida albicans strain W0-1.applutum-like sequence protein 51 (ALS1) gene, ALS1-1 allele, complete cds | Candida albicans | 551 | 551 | 97% | 4e-152 | 85.32% | 4323 | HM164053.1 |
| Candida albicans cell-surface adhesin (ALS1) gene, ALS-large allele, complete cds | Candida albicans | 551 | 551 | 97% | 4e-152 | 85.32% | 4152 | AY227440.1 |
| Candida albicans SC5314.Als5z (ALS5) partial mRNA | Candida albicans | 551 | 551 | 97% | 4e-152 | 85.32% | 4044 | XM_712981.2 |

Figure 5: The identification of query *C. albicans* alignment with NCBI GenBank.

Table 3: Distribution of *C. albicans* according to NBLAST in GenBank

| Sample | Query % | Identic Number % | Genbank | Country |
|----------|---------|------------------|-------------|---------|
| | 100 | 100 | JQ034421.1 | China |
| | 100 | 100 | JQ307472.1 | China |
| | 100 | 100 | JQ307471.1 | China |
| | 100 | 100 | XM_712984.2 | USA |
| | 100 | 100 | OR664373.1 | USA |
| | 100 | 100 | OR664374.1 | USA |
| | 100 | 100 | JQ307470.1 | China |
| PQ328951 | 100 | 99.82 | AY445055.1 | USA |
| PQ328952 | 100 | 99.82 | JQ307473.1 | China |
| PQ328953 | 100 | 99.82 | L25902.1 | USA |
| PQ328954 | 100 | 99.82 | JQ307478.1 | China |
| | 97 | 97 | AF068866.1 | USA |
| | 94 | 100 | CP115803.1 | India |
| | 97 | 85.50 | JQ307474.1 | China |
| | 97 | 85.50 | AF025429.1 | USA |
| | 97 | 85.32 | AY227439.1 | USA |
| | 97 | 85.32 | HM164053.1 | USA |
| | 97 | 85.32 | AY227440.1 | USA |
| | 97 | 85.32 | XM_712981.2 | USA |

Discussion

Candida albicans is an opportunistic pathogen that causes superficial and systemic mycoses in animals, including poultry (30,31). Excessive and incorrect use of medications, hormones, and immunosuppressants in the last few years, in addition to contamination of feed, has led to an increase in the thrush rate in animals and humans (32,33). All isolates used in this study were confirmed in

chromogenic agar gave a green color this result is in agreement with (34,35). The germ tube formation test is a rapid and reliable method for diagnosing *C. albicans*. This test is highly specific for *C. albicans*, with a specificity of approximately 99% (36).

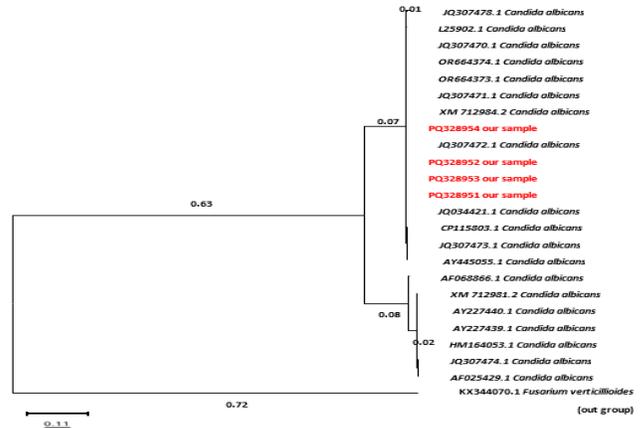


Figure 6: Phylogenetic tree of *Candida albicans* from Iraq (Red color). The phylogenetic tree was constructed using the Maximum Likelihood method based on the Tamura-Nei model in MEGA11 software and bootstrap analysis with 1000 re-samplings. Partial DNA sequences of the gene ALS1 were used as input data.

Many studies have focused on the molecular aspect of *Candida* isolated from humans and rarely considered isolates taken from animals, including birds, where it is necessary to focus on distribution and genetic diagnosis to know the epidemiology (37-39). When using molecular detection of the isolates under study, all isolates showed amplification of the (*18S rRNA* gene) with a molecular weight of 415 bp, which is consistent with (26,33). The molecular investigation used provided more sensitive and specific results (10).

C. albicans possesses several virulence factors that aid in adhesion and invasion of the host tissue (40). The results showed the presence of the *ALS1* gene at a rate of 66%, as many studies have shown its presence at a similar percentage (21,31), the *ALS* genes are the largest family responsible for adhesion, as they include a group of genes that interact with the host cells to enable them to anchor and stabilize (41,42).

Hyphal cells play a fundamental role in the growth and spread of *Candida*, as they invade cells and also damage the tissues. The genes responsible for the production of hyphae are *HWP1*, *ALS1*, and *HGC1* (43,44). The current study showed that all tested *C. albicans* isolates did not contain the *HWP1* gene; this result may be due to the limited number of isolates studied and the methods used (17). This gene is also associated with adhesion, and regulated during biofilm formation (10,45), this result is similar to many studies that showed that at least 195 isolates were negative out of 206 isolates for this gene (21). Earlier studies have revealed that

HWP1 is not expressed during the growth phase of yeast but is highly expressed in hyphal form and germ tube (46). The defects of biofilm formation that are associated with the *HWP1* mutant in vitro and in vivo are completely in agreement with the idea that expression of *HWP1* is specific in hyphae related to biofilm formation (47). Some studies emphasized that *C. albicans* isolated from birds have already been reported in human infections and that these birds and chickens could be considered sources of infection for other animals, and for people as farmers and veterinarians (48).

Conclusion

C. albicans possesses the *ALS1* gene, which is found in a high ratio in the isolates under study, and the *ALS* genes are the largest family responsible for adhesion, interaction with the host cells to enable them to colonize and stabilize, in the outcome participate in the pathogenicity of this yeast. While the tested *C. albicans* isolates did not contain the *HWP1* gene.

Conflict of interest

The authors declare that there is no conflict of interest.

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التحليل الجزيئي لبعض جينات الالتصاق في المبيضات البيضاء المعزولة من الدجاج المصاب بمرض القلاع

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الخلاصة

تسبب المبيضات البيضاء التهاباً في التجويف الفموي للدجاج بسبب امتلاكها لبعض مورثات الضراوة، والتي تمكنها من غزو الخلايا الظهارية للنفخ. هدفت هذه الدراسة إلى التعرف الجزيئي على المبيضات البيضاء المعزولة من الدجاج المصاب بداء المبيضات الفموي (القلاع) والكشف عن بعض مورثات الالتصاق مثل المورث ALS1 والمورث HWP1. خضعت للدراسة الحالية ثمانية عشر عزلة من المبيضات البيضاء المعزولة من الدجاج المصاب بداء المبيضات الفموي في دراسة سابقة أجريت في كلية الطب البيطري / جامعة الموصل. تم إجراء التشخيص الجزيئي باستخدام تفاعل البلمرة المتسلسل. تم استخدام كل من المورثات 18S rRNA و ALS1 و HWP1 للكشف عن المبيضات البيضاء ومورثات الضراوة الخاصة بها. أظهرت الدراسة أن جميع عزلات المبيضات البيضاء أعطت نواتج تضخيم للجين 18S rRNA عند الوزن الجزيئي ٤١٥ زوج قاعدي، بينما أعطت ٦٦٪ من العزلات ناتج تضخيم عند الوزن الجزيئي ٥٧٧ زوج قاعدي للمورث ALS1 في حين أعطت جميع العزلات نتاج سلبية للمورث HWP1. تم تسجيل أربع عزلات من المبيضات البيضاء في البنك العالمي للجينات بالأرقام التسلسلية PQ328951 و PQ328952 و PQ328953 و PQ328954. أظهرت جميع العزلات تشابهاً بنسبة ٩٧-١٠٠٪ مع عزلات المبيضات البيضاء العالمية في كل من دول الصين والولايات المتحدة الأمريكية والهند. وخلصت الدراسة إلى أن العزلات قيد الدراسة تمتلك نسبة عالية من مورث الالتصاق ALS1، مما يساهم بشكل كبير في قدرتها الإراضية في الدواجن.