Study the effect of some physical factors on three isolations of *Candida albicans* isolated from Iraqi patients

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ABSTRACT

Background: The *Candida* spp. is considered an opportunistic fungi, as the infection increases when the immune system is weakened due to the exposure of the host to diseases or as a result of diabetes, immunodeficiency, cancerous diseases, or organ transplantation. **Methods**: The phenotypic and microscopic diagnosis methods for three isolates of *Candida albicans* isolated from different hospitals in Iraq were conducted along with the Vitek2 Compact System, then the diagnosis of isolated yeasts was confirmed based on PCR technology. The physical factors study was conducted to show the effect of temperature, pH concentration, and CO_2 ratio on the three isolates of *Candida albicans*. **Results**: The three isolates contained one single bundle of extracted DNA. The PCR products using the fungal primer pair (ITS1, ITS4) showed the production of a single band for the three isolates of *Candida albicans* of 535bp. While the effect of high temperature on isolates, the best rate of growth was at 37°C, whereas isolate K8 recorded the highest growth rate then isolate B17 and T5, and no growth was recorded for the three isolates at 45°C. As for the effect of pH, the results showed that the best growth was at pH 6.2, where the highest growth rate was in isolate K8, followed by isolate T5 and then isolate B17. The results showed that a high percentage of CO_2 harmed the growth rate; the best growth rate for all isolates was at a concentration of 0.03% of carbon dioxide. The highest growth rate was recorded in isolates K8, followed by B17 and then T5, while the lowest growth rate of isolates was at a concentration of 15% of carbon dioxide, as isolates recorded K8, followed by B17, then T5. **Conclusions**:In this study we found that Candida albicans has a gene expression of 535bp and there were effects of some physical factors on three isolates of pathogenic *Candida albicans*.

Keywords: *Candida albicans*, PCR, Temperature, pH, CO_2.

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1- INTRODUCTION

Candida spp. is considered an opportunistic fungus, as the infection increases when the immune system is weakened due to the exposure of the host to diseases or as a result of diabetes, immunodeficiency, cancerous diseases, or organ transplantation (1,2,3) *Candida albicans* is the most common species in the ability to cause pathological infections, although some cases of other *Candida* species have been recorded. Its colonies are characterized by being cream-colored or white, have a yeast smell, and are large and round in shape (4,5,6). It takes about 48 hours for growth and the appearance of the colonies is a wrinkled or shiny, dry form (7). The *Candida albicans* has a dimorphism, as it grows in a yeasty spherical or filamentous shape depending on changes in temperature, pH, components of the medium, and the amount of moisture, It was noticed that the yeast form of *Candida* spp. grows in an acidic medium when incubated at a temperature of less than 35°C while the filamentous

form requires growth in inorganic nitrogenous media in addition to starchy materials such as Potato Dextrose Agar and Corn meal Agar, These media have a pH of 6.5 or more and a temperature between 20-40°C (8). As (9) indicated the importance of the transition ability of yeast from the yeast form to the filamentous form in the events of infection in the host, because it helps the yeast to stick to cells and resist the defensive macrophages in the body As well as contributes to the mechanical stress on the tissue. The main characteristic that *Candida* possesses in causing disease is the possibility of transition from a spherical or oval Yeast form to a hyphal form, as well as the formation of biofilm, which goes through a series of developments that lasts about 24-48 hours (10). The first stage in which the transformation from yeast to the filamentous form begins is the appearance of the germ tube, with a pH of 2.5-7.5 and a temperature of 37 °C (11). Previous studies indicated that the temperature at which the cells grow may affect the fungus *Candida albicans*, as the cells that grew at room temperature produced germ tubes in abundance, making them more likely to escape from ingestion by the phagocytic cells, as a result rendering them more virulent (12). The effect of growth in an acidic environment works on a structural modification of the innate cell wall, and these disorders in the cell wall lead to an increase in the activity of the innate immune response, which contributes to immune diseases (13). The ability to sense and adapt to changes in carbon dioxide levels is of great importance to all living organisms in general and fungi in particular, as carbon dioxide is one of the main determinants involved in basic biological processes such as virulence, growth, and the ability to form, *Candida albicans* can directly sense the rise of carbon dioxide by adenylyl cyclase, which affects the promotion of growth in the fungal hyphae (14). *Candida albicans* takes advantage of carbon dioxide signals to promote the formation of biofilms inside the host's body, In addition, biofilms grown in anaerobic conditions with high amounts of carbon dioxide show high resistance to azoles and enhance glucose uptake to support rapid growth, as observed in Sef1-dependent iron scavenging, also the lack of oxygen positively affects some important biological processes of the fungus, such as the formation of chlamydial spores and the stability of mating opaque cells (15).

This study aimed to find out the effect of some physical factors on three isolates of pathogenic *Candida albicans.*

2- MATERIAL AND METHODS

2.1. Sample collection

30 samples were taken from inpatient patients, 10 samples each from Yarmouk Hospital in Baghdad, Tikrit Hospital in Salah al-Din, and Al- Kut Hospital to obtain some isolates of *Candida albicans*.

2.2. Morphological and microscopic examination:

The Sabouraud Dextrose Agar culture media was used for the initial isolation of *Candida* spp. and incubated at 37°C. For 48 hours, one of the colonies that were developing was taken on the SDA culture media, and then a drop of blue Lactophenol dye was added to it and observed under the microscope.

2.3. Formation Test Germ tube

Part of the colony was taken and put in a sterile test tube containing 0.5 ml of serum, and incubated at 37°C for 2-4 hours (16).

2.4. Chlamydospore Production Test

Cornmeal Agar was inoculated with a colony of *Candida* spp/ and incubated at 25°C and monitored for 4-6 days (17).

2.5. Diagnosis by Chromogenic agar culture media

The isolates were incubated at 37 ° C for 48-72 hours after being cultured in Chromogenic agar media and determined the *Candida albicans* by color (18).

2.6. Biochemical Identification

Clinically important *Candida albicans* has been precisely diagnosed with the Vitek2 device, according to the manufacturer's instructions Biomerieux U.S.A. (19).

2.7. Measuring the concentration and purity of the extracted DNA from Candida albicans

A Nanodrop spectrophotometer was used to measure the concentration and purity of DNA extracted from samples, by reading the absorbance with a wavelength ranging between 260-280 nm. The extracted DNA purity was measured by the equation: DNA purity = OD260 / OD280 Table (1,2,3)
OD = optical density.

Table (1) .primers used in the study

The size	temperature	sequence	Initiator name
500-700bp	55TM	5'-TCCGTAGGTGAACCTGCGG-3'	ITS1 F
500-700bp	55TM	5'-TCCTCCGCTTATTGATATGC-3'	ITS4R

Table (2) .PCR reaction mixture used in the study

Master mix components	ML/Volume
Master Mix Easy Taq ® RPC Super Mix	12.5
Forward primer	1
Reverse primer	1
DNA	3
Nuclease Free Water	7.5
Total volume	25

Table (3) .PCR technology

Course	Second minute	Temperature C°	steps
1	05.00	95	Initial denaturation
30	00.30	95	denaturation
30		59	Annealing
30		72	Extension
1	07.00	72	Final extension
	10.00	4	Hold

2.8. Preparation of the dilutions of the three isolates of Candida albicans

Serial dilutions of *Candida albicans* isolates in distilled water were prepared then 1ml from each concentration was placed in a dish containing SDA medium and incubated in the incubator for 48 hours at a temperature of 37°C (three replicates for each dilution). The best growth dilution for the three isolates was 10^6 cells/ml, which was adopted in the study of physical factors.

2.9. Study of the effect of some factors on the growth of isolates of Candida albicans

2.9.1.. Temperature effect test

Add1000 microliters of colony diluent at a concentration of (10^6) cells/ml distilled water were taken for each of the three isolates and planted in the medium of (SDA) and placed in the incubator at different temperatures ranging between (25, 30, 45) C°, and a control dish was cultured from each one of the three isolates at a temperature of 37°C for a period of 24 hours, three replicates were used for each isolate and temperature (20).

2.9.2. pH test

Add 1000 microliters of the colony diluent at a concentration of (10^6) cells/ml of distilled water were taken for each of the three isolates and cultured each time with a medium with a certain pH value. The values ranged between (4, 5.5, 8, 9) and then the Petri dishes were incubated at a temperature of 37°C for 24 hours (20).

2.9.3. Test the growth in different proportions of CO_2

Add 1000 microliters at a concentration of (10^6) cells/ml distilled water were taken for each of the three isolates in Petri dishes containing SDA and placed in the Heraeus B5060 EK- CO_2 carbon incubator under a fixed and controllable carbon dioxide concentration at different concentrations of carbon dioxide in the air ranging between (5, 10, 15) at a temperature of 37 °C and for a period of five days, with a control dish being planted for each of the three isolates by placing it in an incubator containing the normal atmospheric concentration of CO_2 (0.03) . The passage of the specified period and the results were recorded (21).

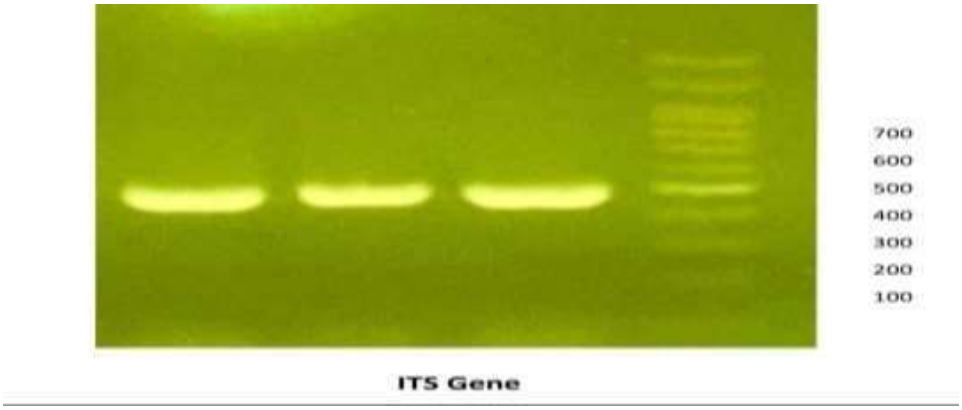
2.10. Statistical Analysis

The Statistical Analysis System program was used to detect the effect of different factors on study parameters. Chi-square test was used to significantly compare percentages in this study. There were no significant differences at $P \leq 0.05$.

3- RESULTS

It was observed by examining the morphology of Candida spp. colonies on SDA medium, that this species appears as creamy white, convex colonies, under the microscope the cells appeared spherical to oval or single long and budding with occasional pseudohyphae *C. albicans* had demonstrated its ability to form a germ tube, a diagnostic characteristic of this species. They appeared oval or spherical with greenish-blue borders under the microscope after staining with the blue-colored lactophenol dye as a result of the accumulation of gram stain on the positive wall due to the presence of a peptidoglycan layer that can retain this dye. The isolated *Candida* spp. was diagnosed by growing it on chromium agar, in which *C. albicans* appeared in light green. To confirm diagnosis Vitek2Compact system was used and gave positive results for *C. albicans.*

3.1



Figure(1). Electrophoresis using agarose gel with a concentration of 1.5% showing PCR results for the analysis of *Candida albicans* ITS1-ITS4

Table (4). Shows three isolates of *Candida albicans* resulting from the polymerase chain reaction

Candida albicans	**isolation number**	**size (ITS1-ITS4)bp**
Candida albicans	**B17**	**535**
Candida albicans	**T5**	**=**
Candida albicans	**K8**	**=**

3.2. Physical factors affecting the growth of Candida albicans:

3.2.1. Test growth at different temperatures

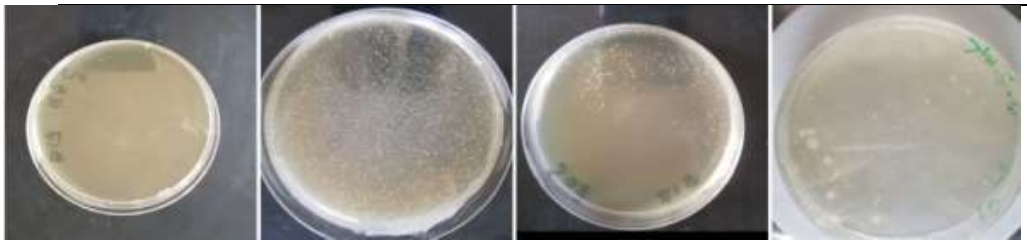
Candida albicans is one of the most important species of yeasts and the most common as it is an opportunistic fungus that is pathogenic to humans, its ability to switch between the yeast form and the filamentous

Form is one of the important virulence factors as it makes the fungus tolerate high temperatures, which reach 37°C. The results of the study showed that for the three isolates at different temperatures, at a temperature of 25°C the largest number of colonies growth in isolate B17 was $46.3*10^7$ cells /ml Fig.(2), then isolate K8 was $45*10^7$ cells/ml and the least growth was for isolate T5 which amounted to $12*10^7$ cell/ml. at a temperature of 30°C, the highest growth rate in isolate K8 was $54.5*10^7$ cells/ml Fig .(3)followed by isolate B17 which reached $6.8*10^7$ cells/ml and the lowest growth rate in isolate T5 recorded $1.37*10^7$ cells/ml and at a temperature of 37°C (degrees of Control), The highest growth rate of isolate K8 of all isolates was $75*10^7$ cells/ml followed by isolate B17 which reached $52.4*10^7$ cells/ml and the least growth rate for colonies was in isolate T5 which amounted to $48.5*10^7$ cells /ml Fig .(4). at a temperature of 45°C no growth was recorded for the three studied isolates, the control temperature showed the highest growth rate of colonies in the three isolates, and the results showed significant differences below the level of Probability $P \leq 0.05$ As shown in Table (5).

Table (5). Shows the number of colonies cells/ml for the three *C.albicans* isolates under the influence of different temperatures

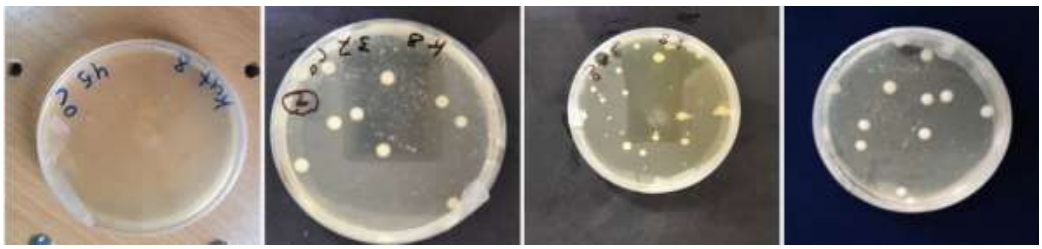
Isolates name	Different temperatures			
	25°C	**30°C**	**37°C control**	**45°C**
B17	$46.3*10^7$	$6.8*10^7$	$52.4*10^7$	0
T5	$12*10^7$	$1.37*10^7$	$48.5*10^7$	0
K8	$45*10^7$	$54.5*10^7$	$75*10^7$	0

***p value** Pearson's chi-square = $\chi^2 = 0.000$, Pearson's correlation coefficient = 0.664, Spearman's coefficient = 0.771



45°C **37°C** **30°C** **25°C**

Figure (2).Shows the growth of colonies of isolate B17 at different temperatures and pH=6.2 for 24 hours



45°C **37°C** **30°C** **25°C**

Figure (3).Shows the growth of colonies of isolate K8 at different temperatures and pH=6.2 for 24 hours

45°C **37°C** **30°C** **25°C**

Figure (4). Shows the growth of colonies of isolate T5 at different temperatures and pH=6.2 for 24 hours

3.2.2. pH effect

The results of Table (6) showed the effect of different concentrations of pH on colony growth of the three isolates of *C. albicans*. The table shows that isolate B17 can grow at all used pH levels, and isolate K8 recorded the highest growth ever at pH 6.2 compared with the rest of the isolates and other pH values. The pH degree of 6.2 (control) recorded the highest growth level in isolate K8 reaching $74.6*10^7$ cells/ml as illustrated in Fig .(5), followed by isolate B17 which was $52.3*10^7$ cells/ml as in Fig. (6), the least developed was the T5 isolate, which amounted to $48.4*10^7$ cells/ml. As for the pH degree of 9, isolate T5 showed the highest growth, which reached $68.0*10^7$ cells /ml as shown in Fig .(7), followed by isolate B17 which reached $22.6 * 10^7$ cells/ml, the lowest growth was isolate K8 $0.5*10^7$ cells/ml. At pH 8, the growth rate of isolates T5 and B17 was equal and reached $27*10^7$cells/ml, while isolate K8 did not show any growth, while at pH 5.5 the growth rate was low, as isolate T5 recorded a growth rate of $6.54*10^7$ cells/ml, Whereas the B17 isolate was $1.013*10^7$ cells/ml, while the K8 isolate did not record any growth. At pH 4, isolate B17 showed a very low growth rate of $1.020*10^7$ cells/ml, and no growth was recorded in the other two isolates. The results showed that there are differences Significant under the probability level of $P \leq 0.05$.

Table (6). Shows the number of colonies cells/ml for the three *C. albicans* isolates under different pH concentrations.

Isolates name	Different pH concentrations				
	4	**5.5**	**6.2 control**	**8**	**9**
B17	$1.020*10^7$	$1.013*10^7$	$52.3*10^7$	$27*10^7$	$22.6*10^7$
T5	0	$6.54*10^7$	$48.4*10^7$	$27*10^7$	$68.0*10^7$
K8	0	0	$74.6*10^7$	0	$0.5*10^7$

***p value** Pearson's chi-square = $\chi^2 = 0.000$, Pearson's correlation coefficient = 0.133, Spearman's coefficient = 0.074

pH=4 **pH=5.5** **pH=6.2** **pH=8** **pH=9**

Figure (5). Shows the growth of colonies of isolate K8 at different pH concentrations and incubated at 37°C for 24 hours.

pH=4 **pH=5.5** **pH=6.2** **pH=8** **pH=9**

Figure (6). Shows the growth of colonies of isolate B17 at different pH concentrations and incubated at 37°C for 24 hours.

pH=4 **pH=5.5** **pH=6.2** **pH=8** **pH=9**

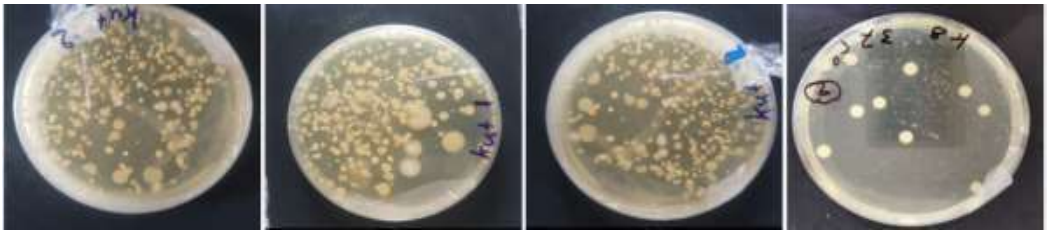
Figure (7). Shows the growth of colonies of isolate T5 at different pH concentrations and incubated at 37°C for 24 hours.

3.2.3. Effect of growth in different concentrations of CO_2

The ability of the three isolates of *Candida albicans* to change the cell shape and growth rate of colonies was studied at three concentrations of CO_2 (15,10,5) %compared to the control atmospheric concentration of 0.03% (Table 7). At 0.03% CO_2 isolate K8 recorded the highest growth rate of $76.7*10^7$ cells/ml as shown in Fig.(8), followed by isolate B17 which reached $50.5*10^7$ cells/ml as illustrated in Fig .(9), while isolate T5 recorded the lowest growth rate of $48.3*10^7$ cells/ml as in Fig .(10), however, Isolate B17 recorded the highest growth rate at 5% CO_2 concentration, reaching $47*10^7$ (cells/ml) followed by isolate T5 which recorded $40 * 10^7$ cells/ml, the least growth was for isolate K8 reaching $33.4*10^7$ cells/ml. but at a concentration of 10%, the highest growth rate was in isolate T5 which reached $40.2*10^7$ (cells/ml), followed by a small difference in isolate B17 which recorded $40*10^7$ cells/ml, the lowest growth rate was in isolate K8 which reached $26.1*10^7$ cells/ml. the results of growth in the last concentration recorded 15%, the highest rates of growth before isolate T5 was $48 * 10^7$ cells/ml followed by isolate B17 which amounted to $30.8*10^7$ cells/ml and the lowest in the number of colonies was isolate K8, which reached $23*10^7$ (cell/ml). The results showed that there were significant differences under the probability level of $P \leq 0.05$.

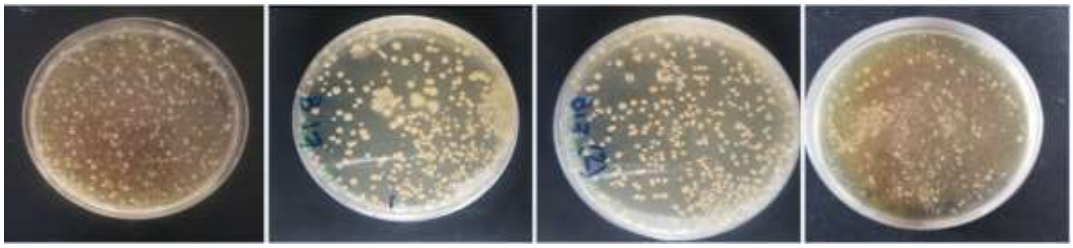
Table (7). Shows the number of colonies (cells/ml) for the three *C. albicans* isolates under the influence of different CO2 concentrations

Isolates name	%0.03 Control	Different CO_2 concentrations %5	%10	%15
B17	$50.5*10^7$	$47*10^7$	$40*10^7$	$30.8*10^7$
T5	$48.3*10^7$	$40*10^7$	$40.2*10^7$	$48*10^7$
K8	$76.7*10^7$	$33.4*10^7$	$26.1*10^7$	$23*10^7$
***p value**	Pearson's chi-square = χ^2 = 0.000, Pearson's correlation coefficient = 0.593, Spearman's coefficient = 0.715			



0.03% **5%** **10%** **15%**

Figure (8). Shows the growth of colonies of isolate K8 at different concentrations of CO_2 and incubated at 37°C for 24 hours.



0.03% **5%** **10%** **15%**

Figure (9). Shows the growth of colonies of isolate B17 at different concentrations of CO_2 and incubated at 37°C for 24 hours.

0.03% **5%** **10%** **15%**

Figure (10). Shows the growth of colonies of isolate T5 at different concentrations of CO_2 and incubated at 37°C for 24 hours.

4- DISCUSSION

It was observed by examining the morphology of *Candida* spp. colonies growing on cultured media in SDA medium, this species appears as creamy white, convex, and fine textured colonies, This result is consistent with (22). *Candida* spp. colonies have the same characteristics that have been mentioned, in addition to the cells being spherical to oval or single-long and budding with occasional pseudohyphae and this is in agreement with (23) (24). *C. albicans* has demonstrated its ability to form a germ tube, a diagnostic characteristic of this species in agreement with (25) (26). During the microscopic examination of the colonies of the *Candida* spp. after adding the blue-colored lactophenol dye, the results were oval or spherical with greenish-blue borders as a result of the accumulation of gram stain on the positive wall due to the presence of a peptidoglycan layer that has the ability to retain this dye (27). The isolated *Candida* spp. was diagnosed by growing it on chromium agar, in which *C. albicans* appeared in a light green color (20).

4.1. Molecular diagnosis by Polymerase chain reaction PCR technique:

The diagnosis was made using the universal primer pair for fungi (ITS1-ITS4), which is based on the amplification of the Internal transcribed space (ITS) region, which contains (ITS1-5.8S-ITS4) as shown in Table (3). It was found that an amplicon of 535 bp which is characteristic of *Candida albicans* was produced and these results are consistent with what was mentioned (28,29).

4.2. Physical factors affecting the growth of Candida albicans:

4.2.1. Test growth at different temperatures:

The results of the study showed that for the three isolates at different temperatures, Fig (2), Fig (3), Fig (4). These results are consistent with what was mentioned by (20). Those who mentioned that the highest growth rate is at a temperature of 37 °C, as this temperature stimulates the *candida albicans* to change its shape from the spherical or oval yeast shape to the filamentous shape, which helps to move easily between the cells of the body and the incidence of infection.

The study showed that there is a relationship between temperature and the ability of cells to adhere to the surface of epithelial cells, the highest adhesion ability was for colonies that were growing at a temperature of 25°C, the higher the growth temperature, the lower the ability of Candida to attach, also blastospores that grow in a medium at a temperature of 25°C have a higher ability to adhere to vaginal epithelial cells in much greater numbers than those isolated in cultures grown at a temperature of 37 °C (30).

4.2.2. pH effect:

The results of Table (6) Fig (5) Fig (6) Fig (7) showed the effect of different concentrations of pH on colony growth of the three isolates of *C. albicans.* the table shows that isolate B17 has the ability to grow at all used pH levels, The previous results showed that the highest rate of growth in the base medium is more than in the acidic medium, and this result is consistent with (20), who mentioned that the highest growth rate ranged between pH 1.5-7 and at a temperature of 37 °C.

Candida albicans is one of the causes of opportunistic fungal diseases for humans, as it can colonize and infect areas of varying pH such as the acidic membrane of the vagina, the oral cavity, and the stomach membrane, the effect of growth in an acidic environment works on a structural modification of the innate cell wall, and these disorders in the cell wall lead to an increase in the activity of the innate immune response, which contributes to immune diseases (13). Shifts in pH can be the cause of changes in the activity of extracellular decomposition, as the host cell membrane is modified in an acidic environment, the hydrolysis of the protein on the surface of the *candida* cell leads to a change in hydrophobicity, which leads to the ability of the cell to stick, previous study showed that there is a negative relationship between the pH and the ability to stick (31). The mucous layers colonized by *Candida albicans* differ greatly in the surrounding pH, as the functions and properties of the wall proteins depend on the pH, and *C. albicans* can adapt its wall protein to the pH outside, most fungi live in moderately acidic environments and the modification of pH is one of the means of escaping the defensive response of the host's immune system and facilitating reproduction or stimulating the destruction of host tissues, many fungi use specific carbon or nitrogen metabolism pathways to provide ammonia that is released from the cell to raise the pH of the environment in which they live, environments vary in pH ranging from basic in the intestines moderate in the blood and little acidity on the surface of the skin up to the highest acidity in the stomach (32).

4.2.3. Effect of growth in different concentrations of CO_2:

The ability of the three isolates of *Candida albicans* to change the cell shape and growth rate of colonies was studied at three concentrations of CO_2 (15,10,5%) compared to the control atmospheric concentration of 0.03% (Table 7) Fig (9).

The results showed an increase in the growth rates of colonies in the three isolates when the concentration of CO_2 decreased, as the ability of the ovaries to grow increased, as well as the change in the shape of the colonies in the higher concentrations of CO_2. These results are consistent with (32), as they showed that changes in the environment *Candida* leads to the remodeling of the cell wall and one of the most important sources that affect the formation of the cell wall is the source of carbon and oxygen deficiency, *Candida albicans* adapt to the lack of oxygen by increasing its metabolic activity.

5. CONCLUSIONS

The Candida spp. is considered an opportunistic fungi, as the infection increases when the immune system is weakened due to the exposure of the host to diseases or as a result of diabetes, The three isolates Candida albicans from the isolated from Iraqi diabetic patients, contained one single bundle of extracted DNA. The PCR products using the fungal primer pair (ITS1, ITS4) showed the production of a single band for the three isolates of Candida albicans of 535bp. The physical factors study was conducted to show the effect of temperature, the best rate of growth was at 37°C. pH concentration, that the best growth was at pH 6.2 and CO_2 ratio on the three isolates of Candida albicans. The best growth rate was at a concentration of 0.03% of carbon dioxide, for all isolates.

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دراسة تاثير بعض العوامل الفيزيائية في ثلاث عزلات من *Candida albicans* معزولة من مرضى عراقيين

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الخلاصة

خلفية البحث: تعتبر المبيضات .Candida Spp فطريات انتهازية، حيث تزداد العدوى عند ضعف الجهاز المناعي بسبب تعرض المضيف للأمراض أو نتيجة مرض السكري أو نقص المناعة أو الأمراض السرطانية أو زراعة الأعضاء. **طريقة العمل**: تم تشخيص الشكل المظهري والمجهري لثلاثة أنواع من المبيضات Candida albicans المعزولة من مستشفيات مختلفة في العراق، باستخدام نظام فايتك، ثم تم تأكيد تشخيص الخمائر المعزولة بناء على تقنية تفاعل البوليميريز المتسلسل. أجريت دراسة العوامل الفيزيائية لإظهار تأثير درجة الحرارة وتركيز الرقم الهيدروجيني ونسبة غاز ثنائي اوكسيد الكاربون على العزلات الثلاث للمبيضات .Candida albicans **النتائج:** أظهرت النتائج تضخيم الحمض النووي لعزلات الخمائر. واحتوت العزلات الثلاث على حزمة واحدة من الحمض النووي المستخلص. أظهرت منتجات تفاعل البوليميريز المتسلسل باستخدام زوج البادیء الفطري (ITS1 ، ITS4) إنتاج ، وان Candida albicans لها تعبير جيني 535 زوج قاعدي ، فقد كان أفضل معدل نمو عند 37 درجة مئوية، في حين سجلت عزلة K8 أعلى معدل نمو ثم عزلة B17 و .T5 ولم يتم تسجيل أي نمو للعزلات الثلاث عند 45 درجة مئوية. أما بالنسبة لتأثير الرقم الهيدروجيني فقد أظهرت النتائج أن أفضل نمو كان عند الرقم الهيدروجيني 6.2 حيث كان أعلى معدل نمو في عزلة K8 يليها عزلة T5 ثم عزلة B17.أظهرت النتائج أن نسبة عالية من غاز ثنائي اوكسيد الكاربون كان لها تأثير سلبي على معدل النمو ، وكان أفضل معدل نمو لجميع العزلات عند تركيز 0.03٪ من ثاني أوكسيد الكربون. وسجلت أعلى نسبة نمو في العزلات K8 تلتها B17 ثم T5 بينما كان أقل معدل نمو للعزلات عند تركيز 15٪ من ثاني أكسيد الكربون حيث سجلت العزلات K8 تلتها B17 ثم .T5 **الاستنتاج:** في هذه الدراسة وجدنا أن Candida albicans لها تعبير جيني 535 bpوكانت هناك تأثيرات لبعض العوامل الفيزيائية على ثلاث عزلات من Candida albicans المسببة للأمراض.

الكلمات المفتاحية: خميرةCandida albicans ، تفاعل البوليميز المتسلسل، درجة الحرارة، الرقم الهيدروجيني، ثنائي اوكسيد الكربون .