# African Journal of Advanced Pure and Applied Sciences (AJAPAS)

*Online ISSN: 2957-644X*

Volume 2, Issue 3, July-September 2023, Page No: 180-186

Website: *https://aaasjournals.com/index.php/ajapas/index*

||Arab Impact factor 2022: 0.87|| SJIFactor 2023: 5.689|| ISI 2022-2023: 0.557


# Molecular identification of *Candida sp.* Whish isolated from patients and effect pH , temperature and carbon source in growth


Marwa Hussein Hummadi [1 *], Mohammad Ibrahim Khalil [2]

[1,2] Department of Environmental Sciences, College of Environmental Science and Technology, University of Mosul, Iraq

[*]*Corresponding author: marwa.21evp5@student.uomosul.edu.iq*



| Received: June 13, 2023 | Accepted: July 21, 2023 | Published: August 02, 2023 |
|---|---|---|



**Abstract:**

The study aimed to collecting 100 clinical samples of fungi of the genus Candida spp. from patients with different ages and both genders who visit hospitals in Mosul city. 70 samples from the vaginal area and 30 samples from the mouth area, using sterile cotton swabs. Four species of yeast (*C. albicans, C. tropicalis, C. glabrata*, and *C. krusei*) isolated from the ovary. Tests were conducted to identify the effect of some environmental factors (temperature, pH and some saccharides) that stimulate the filamentous growth of yeast strains. The results showed that the temperature of 40°C and pH 6.5 recorded the highest stimulation of filamentous growth of C. albicans. Whereas the saccharides (sucrose, fructose and mannitol) test was conducted showed that the sucrose medium had the highest stimulation of filamentous growth when isolating *C. albicans*. Molecular identification was confirmed based on variation in the ITS region to confirm the diagnosis of fungal isolates." The globally recognized genetic code was used as an advanced molecular diagnostic level, and four species of *Candida spp*. were recorded*: candida albicans, candida tropicalis, Nakaseomyces sp., and pichia kudriavzevil* at the National Center for Biotechnology (NCBI). The results of the phenotypic diagnostic test were similar to those of the molecular diagnostic test using PCR.



**Keywords**: Molecular identification, *Candida sp*. Carbon source, temperature , pH

**Cite this article as:** M. H. Hummadi, M. I. Khalil, "Molecular identification of Candida sp. Whish isolated from patients and effect pH, temperature and carbon source in growth," *African Journal of Advanced Pure and Applied Sciences (AJAPAS)*, vol. 2, no. 3, pp. 180–186, July-September 2023.

Publisher's Note: African Academy of Advanced Studies – AAAS stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.




# التشخيص الجزيئي لـأنواع المبيضات (Candida sp) المعزولة من المرضى وتأثير الرقم الهيدروجيني ودرجة الحرارة ومصدر الكربون في النمو


مروة حسين حمادي[*1]، محمد ابراهيم خليل[2]

[2, 1] قسم العلوم البيئية، كلية علوم البيئة وتقنياتها، جامعة الموصل، العراق



**الملخص**

هدفت الدراسة إلى جمع 100 عينة سريرية من فطريات جنس المبيضات. من المرضى من مختلف الأعمار وكلا الجنسين المراجعين الى مستشفيات مدينة الموصل. 70 عينة من منطقة المهبل و30 عينة من منطقة الفم باستخدام مسحات قطنية معقمة. تم عزل أربعة أنواع من الخميرة (*C. albicans* ، و *C. tropicalis* ، و *C. glabrata* ، و *pichia kurdiavzevil*) (*C. krusei*) من المبيضات. أجريت اختبارات للتعرف على تأثير بعض العوامل البيئية (درجة الحرارة

ودرجة الحموضة وبعض السكريات) التي تحفز النمو الخيطي لسلالات الخميرة. أظهرت النتائج أن درجة الحرارة البالغة 40 درجة مئوية ودرجة الحموضة pH 6.5 سجلت أعلى تحفيز للنمو الخيطي لفطر *C. albicans*. في حين أنه أظهر اختبار السكريات (السكروز والفركتوز والمانيتول) أن وسط السكروز كان له أعلى تحفيز للنمو الخيطي عند عزل المبيضات البيضاء. تم تأكيد التشخيص الجزيئي بناءً على منطقة ITS لتأكيد تشخيص العزلات الفطرية. "تم استخدام الكود الجيني المعترف به عالميًا كمستوى تشخيص جزيئي متقدم، وتم تسجيل أربعة أنواع من *Candida spp :.C.albicans* ، *C.tropicalis* ، *pichia kudriavzevil (C.krusei), Nakaseomyces sp (C.glabrata)* في المركز الوطني للتقنيات الحيوية (NCBI). وكانت نتائج اختبار التشخيص المظهري مماثلة لنتائج اختبار التشخيص الجزيئي باستخدام PCR.

**الكلمات المفتاحية:** التشخيص ألجزيئي، المبيضات، مصدر الكربون، درجة الحرارة، الرقم الهيدروجيني

## Introduction

Over the past forty years, fungal pathogens have become responsible for increasing the rate of human mortality. The majority of hospital-related infections in the developed countries are caused by Candida spp., particularly Candida albicans. Candida infections can range, in severity, from moderate superficial infections to potentially fatal invasive infections. Candida infections continue to have a significant mortality rate despite the use of antifungals and other therapeutic methods [1,2]. Numerous studies have demonstrated that C. albicans' capacity to spread disease and morphogenesis are closely related. Although C. albicans exhibits a wide range of morphological transitions (such as the formation of chlamydospores, the GUT phenotype, the switching from white to opaque, and the gray phenotype), the yeast-to-hypha transition appears to be a crucial virulence trait [3–7].

The mechanisms of transition between the unicellular yeast form and either the pseudohyphal or hyphal forms, which are frequently referred to as filamentous forms, were therefore of particular interest [8,9]. For instance, the yeast form is necessary for attachment to endothelial cells. and dispersion into the bloodstream [10,11]. The filamentous form, on the other hand, is necessary for tissue invasion and for evading immune cells, resulting in resistance to phagocytosis [12–15].

The capacity to react to environmental cues is a fundamental aspect that fosters C. albicans pathogenicity. various environmental circumstances. One significant illustration of this is how different stimuli, such as serum, an alkaline pH, carbon dioxide (CO2), and N- acetylglucosamine (GlcNAc), cause C. albicans to go from developing as budding cells to creating filamentous chains of pseudohyphal and hyphal cells [16].

## Material and methods

### Sample collection

Yeasts isolates were obtained by taking swabs from the mouth and vagina of the patients infected who are regular visitors of Mosul city hospitals and from both sexes and different ages. So, one hundred (100) samples were, 70 of which were taken from the vagina and 30 from the mouth using sterilized swabs.

### Isolation and identification

The saboruraud Dextrose Agar was used to isolate the types of candida in petri sterilized plates and they were incubated for 48 hours at a temperature degree of 37º C. Then, the Lactophenol cotton blue and gram stain were used to color the isolated yeasts. Also, the chromogenic agar test and API kit was used, which was provided by BioMèrieux France company.

Studying the effect of some factors that stimulate the filamentous growth of some candida spp.

**1. The effect of temperature**

In this test, the Potato Dextrose Agar (PDA) was used, where the samples were taken and cultured in this medium and they were incubated in different temperature degrees (20, 25, 30, 35 and 40) º C for 72 hours.

**2. The effect of pH**

In this experiment, five values of pH (4.5, 5, 5.5, 6 and 6.5) were used in terms of the effect of pH using the PDA medium that was prepared. The agar was distributed in four glass flasks with a volume of (250) ml of the agar in each flask. The pH of media was modified by adding drops of Hydrochloric acid (HCl) or by adding Sodium hydroxide (NaOH) solution under conditions of sterilization. The media (agar) were poured in petri plates with two replicates for each pH value and then the plates were incubated in 40 º C for 48 hours.

**3.The effect carbon sources**
The liquid potato extract, prepared in the laboratory, was used in 250 ml-volume flasks and 20 grams of fructose, sucrose and manitol in each flask. Under the conditions of sterilization, the media were poured into petri plates with two replicates for each type of saccharides and the plates were incubated for 72 hours at a temperature of 40º

**4.Germ tube formation**
A quantity of 0.5-1 of human blood serum was put in a sterilized test tube and then the tube was inoculated with a tiny part of the colony cultured on SDA. Then the tube was incubated at 37 º C for 2-3 hours for the purpose of comparison according to the time periods of growth.

**Molecular identification**
The isolated fungi were cultured in a nutrient broth and the agar was poured in sterilized vials for the purpose of the fungi growth and for the molecular diagnosis.

The molecular diagnosis using the PCR technique
DNA extraction from the yeast samples studied
The Genomic DNA mini Kit for extracting and purifying the DNA was used, which was made by Geneaid company.
Preparation of Agarose Gel and the Electrophoresis of the DNA
Electrophoresis was conducted using the agarose gel according to was mentioned by[17,18]. where this method was used to detect the products of the PCR. The agarose gel was put on the display of the UV Trans Illumination so as to see the DNA bands and also to see the PCR reaction products.

**The PCR reactions**
This method was used to amplify the ITS area in the DNA on which the pairs of the primers ITS1-ITS4 act to amplify the ITS in the yeast using the PCR technique. The presence of the gene in the yeast samples as 4 microliters (100 nanogram) of the template DNA were added and also 1 microliter (10 picompl) of the gene primer was added to the master mix. After that, the reaction tubes were introduced to the Thermocycler to perform the chain reaction using the reaction program as shown in table (1):

**Table (1):** Polymerase chain reaction PCR Program

| Co. | Stage | Temperature | Time | Cycle Cumber |
|---|---|---|---|---|
| 1. | Initial denaturation | 95 | 6 min. | 1 |
| 2. | Denaturation | 95 | 45 sec. | 35 |
| 3. | Annealing | 58 | 1 min. | |
| 4. | Extension | 72 | 1 min. | |
| 5. | Final extension | 72 | 5 min. | 1 |

**Results and discussion**
Thirty (30) isolates of *Candida spp*. that were isolated from the mouth and the vagina were obtained and they were identified depending on the morphological characteristics of the colonies and the microscopic features.

**Isolation and Identification**
The samples were isolated and identified by relying on several tests, including culturing the samples on SDA for 48 hours in the primary isolation as four fungi species of candida spp. were isolated; 14 of which belong to C. albicans and 12 samples belong to *C.krusei*, one sample belongs to *C. tropicalis* and three samples belong to Calibrate. the identification of all the samples was confirmed depending on the diagnostic and biochemical tests in the Chromogenic agar and by using API to discriminate the albicans types.

***The effect of temperature***
The results of the study showed the effect of temperature on the growth of Candida spp. as shown in figure (1). The temperature degree is 40°.

C showed the best filamentous growth of the *C.albican* isolate and that is because the high temperature affects the morphology of the fungus and therefore, stimulates the filamentous growth and leads to the change of the fungus spherical or oval shape to the pathogenic filamentous shape that contributes the easiness of moving between the body cells and inflicting the infection[19]. From the other hand, isolates at low temperature degrees showed a slow growth due to the slowness of metabolism process, which affects the growth rate and the difference in temperature could be stimulating, inhibiting or lethal, while with the rise in temperature the activity of enzymes and protein reaches the peak [20]. The results of the statistical analysis showed that there were significant differences in stimulating the growth according to the different temperature degrees at the level ($p \leq 0.05$).

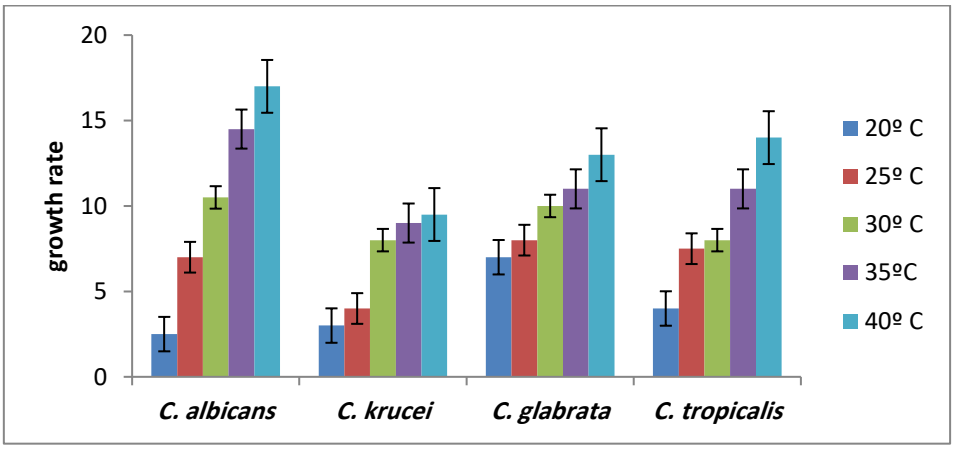


**Figure 1:** The effect of temperature on the growth of Candida spp.

***The of pH effect***

The results of the effect of different values of pH on the filamentous growth of the fungi isolates, as shown in figure (2), showed that C. albicans demonstrated the highest growth with a value pH (6.5) and the lowest growth rate was at the pH value of (4.5). the reason behind that is the isolate C.albicans has adaptation capability with all the values of pH and its response involves the stimulation of the filamentous growth in the pH value that tends to alkalinity, but the low value of pH prevents the growth as filaments and stimulates the growth of the spherical shape of the yeast[21]. Results of the statistical analysis showed that there were significant differences in stimulating the growth according to the value of pH at the level ($p \leq 0.05$).

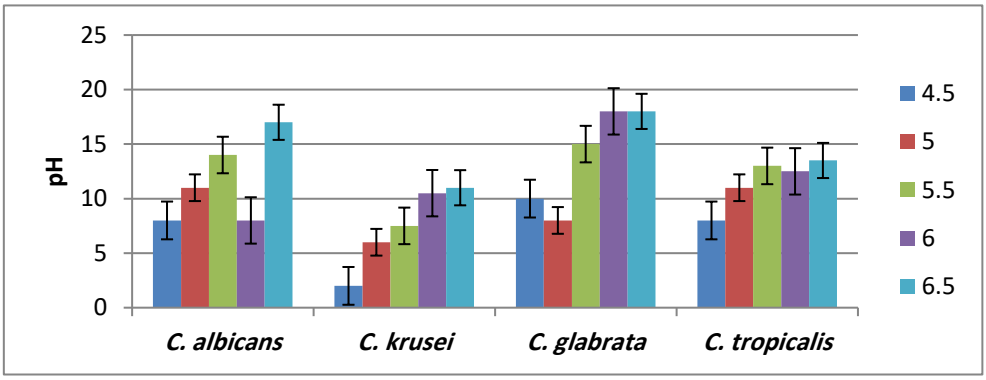


**Figure 2:** The effect of pH on the growth of Candida spp.

**Carbon sources:**

The study of the effect of the carbon source on stimulating the filamentous growth of candida spp. was conducted and the highest growth of isolates was through using the sucrose, fructose and manitol. The best growth was on the medium of the sucrose as candida spp. has the ability to assimilate the different sources of carbons because it possesses metabolism flexibility in metabolism that enables it to change the structure of the cell wall and this develops in it an adaptive response with all the sources of the carbon [22]. From the other hand, mannitol medium recorded the lowest filamentous growth due to a disorder in the mitochondria function that decreases the activity

of the mannitol because of the activity of the hydrogenase enzyme, which is responsible for the metabolism of manitol and so the sugar will not be decomposed throughout the process of metabolism [23].

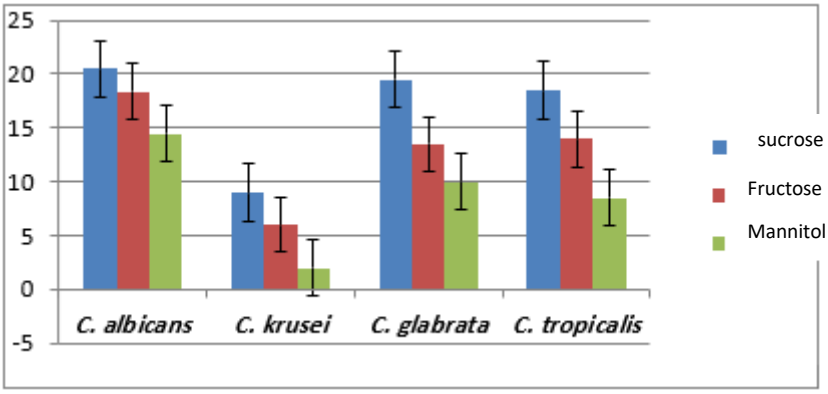


**Figure 3:** The effect of carbon sources on the growth of candida spp**.**

**Serum tests (germ tube)**

Results showed that most of isolates couldn't form germ tube after putting them in a tube that contains blood serum for (2-3) hours except for the *C.albicans* and *C. tropicalis* isolates, which demonstrated their capability for forming the germ tube that is considered a distinguishing feature of the type *C.albicans* in 37º C [24], but in the temperature 40º C *C.albicans* showed a formation of germ growth.

**Molecular identification**

**Extracting the DNA from the yeasts isolated**

The results of extracting the DNA showed that the emergence of pure individual bands is an evidence of the success of the extraction process as shown in the figure below:

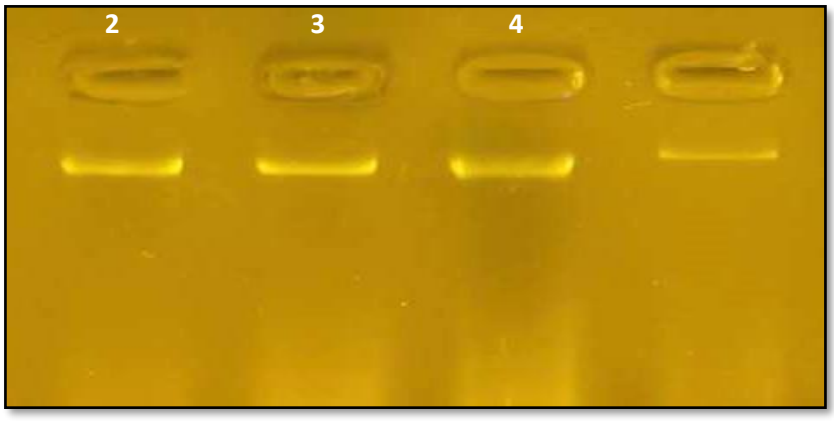


**Figure 4:** Genomic DNA extraction.

**Identification by amplification of ITS region:**

The results of Internal transcribed spacer (ITS) region amplification for the four isolates selected from the yeasts showed important patterns that contributed to the identification of some types of yeasts in question, because it is considered a genetic taxonomical indicator that can be used to study the different fungi variations in this region. It was observed that four bands of purified DNA emerged when the reaction product of PCR (500-700bp) was amplified for the ITS region of the yeast as shown in the figure below:

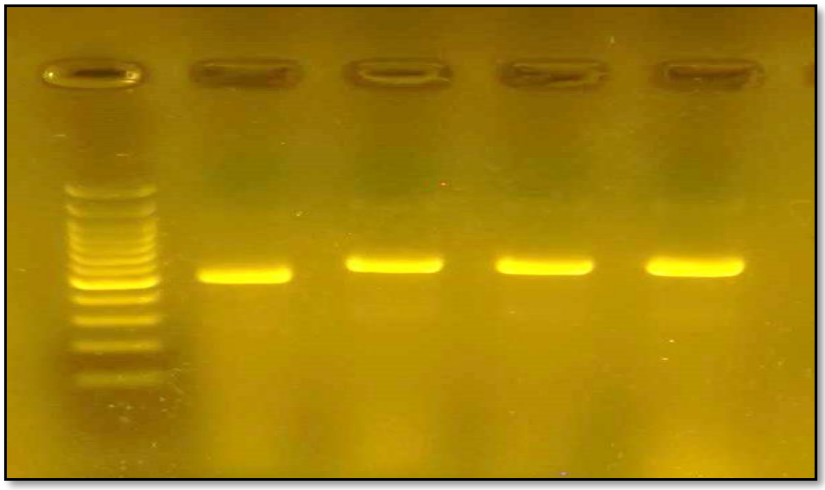

**Figure 5:** The PCR reaction product of ITS region in the 2% agarose gel. The reaction product was (500-700bp).

The results of the reaction were sent to Psomagen USA company to identify the sequence of the nitrogen bases. The results showed the record of four isolates of yeasts in the location NCBI and each isolate has its own international number of registrations as shown in the table below:

**Table 2:** Record fungal insulation on NCBI

| Scientific name of the isolate | International number |
|---|---|
| *Candida tropicalis* | LC768881 |
| *Nakaseomyces sp* | LC768882 |
| *Candida albican s* | LC768883 |
| *Pichia kudriavzevii* | LC768884 |

## Conclusion

In this study concluded that the results of the phenotypic diagnostic test were similar to those of the molecular diagnostic test using PCR and high effect of different environmental effect in candida growth.

## References


[1] B.J. Kullberg, M.C. Arendrup, Invasive candidiasis., N. Engl. J. Med. 374 (2016) 794–795.
[2] M.A. Pfaller, D. Diekema, Epidemiology of invasive candidiasis: a persistent public health problem, Clin. Microbiol. Rev. 20 (2007) 133–163.
[3] A.J.P. Brown, N.A.R. Gow, Regulatory networks controlling Candida albicans morphogenesis, Trends Microbiol. 7 (1999) 333–338.
[4] L. Mukaremera, K.K. Lee, H.M. Mora-Montes, N.A.R. Gow, Candida albicans yeast, pseudohyphal, and hyphal morphogenesis differentially affects immune recognition, Front. Immunol. 8 (2017) 629.
[5] J.C. Nemecek, M. Wüthrich, B.S. Klein, Global control of dimorphism and virulence in fungi, Science (80-.). 312 (2006) 583–588.
[6] B.M. Peters, G.E. Palmer, A.K. Nash, E.A. Lilly, P.L. Fidel Jr, M.C. Noverr, Fungal morphogenetic pathways are required for the hallmark inflammatory response during Candida albicans vaginitis, Infect. Immun. 82 (2014) 532–543.
[7] N. Trevijano-Contador, C. Rueda, O. Zaragoza, Fungal morphogenetic changes inside the mammalian host, in: Semin. Cell Dev. Biol., Elsevier, 2016: pp. 100–109.
[8] F.C. Odds, Candida infections: an overview, CRC Crit. Rev. Microbiol. 15 (1987) 1–5.
[9] P. Sudbery, N. Gow, J. Berman, The distinct morphogenic states of Candida albicans, Trends Microbiol. 12 (2004) 317–324.

[10] C.M. Bendel, D.J. Hess, R.M. Garni, M. Henry-Stanley, C.L. Wells, Comparative virulence of Candida albicans yeast and filamentous forms in orally and intravenously inoculated mice, Crit. Care Med. 31 (2003) 501–507.
[11] S.P. Saville, A.L. Lazzell, C. Monteagudo, J.L. Lopez-Ribot, engineered control of cell morphology in vivo reveals distinct roles for yeast and filamentous forms of Candida albicans during infection, Eukaryot. Cell. 2 (2003) 1053–1060.
[12] L.P. Erwig, N.A.R. Gow, Interactions of fungal pathogens with phagocytes, Nat. Rev. Microbiol. 14 (2016) 163–176.
[13] C. Fradin, P. De Groot, D. MacCallum, M. Schaller, F. Klis, F.C. Odds, B. Hube, Granulocytes govern the transcriptional response, morphology and proliferation of Candida albicans in human blood, Mol. Microbiol. 56 (2005) 397–415.
[14] M.C. Lorenz, J.A. Bender, G.R. Fink, Transcriptional response of Candida albicans upon internalization by macrophages, Eukaryot. Cell. 3 (2004) 1076–1087.
[15] P.J. Rooney, B.S. Klein, Linking fungal morphogenesis with virulence, Cell. Microbiol. 4 (2002) 127–137.
[16] S. Naseem, E. Araya, J.B. Konopka, Hyphal growth in Candida albicans does not require induction of hyphal-specific gene expression, Mol. Biol. Cell. 26 (2015) 1174–1187. https://doi.org/10.1091/mbc.E14-08-1312.
[17] T. Iwata, H. Hattori, H. Chibana, Y. Mikami, Y. Tomita, A. Kikuchi, T. Kanbe, Genotyping of Candida albicans on the basis of polymorphisms of ALT repeats in the repetitive sequence (RPS), J. Dermatol. Sci. 41 (2006) 43–54.
[18] H. Hattori, T. Iwata, Y. Nakagawa, F. Kawamoto, Y. Tomita, A. Kikuchi, T. Kanbe, Genotype analysis of Candida albicans isolates obtained from different body locations of patients with superficial candidiasis using PCRs targeting 25S rDNA and ALT repeat sequences of the RPS, J. Dermatol. Sci. 42 (2006) 31–46.
[19] E. Ezaka, O. Nchedo, E.N. Ugbo, A.B. Adediran, O.E. Ayanda, Effects of Environmental factors on the Growth and Proliferation of Yeasts, Niger. J. Biotechnol. 38 (2021) 166–178.
[20] K. Sánchez-Alonzo, L. Arellano-Arriagada, S. Castro-Seriche, C. Parra-Sepúlveda, H. Bernasconi, H. Benavidez-Hernández, V.L. Campos, K. Sáez, C.T. Smith, A. García-Cancino, Temperatures outside the optimal range for helicobacter pylori increase its harboring within candida yeast cells, Biology (Basel). 10 (2021) 915.
[21] S.G. Nadeem, A. Shafiq, S.T. Hakim, Y. Anjum, S.U. Kazm, Effect of growth media, pH and temperature on yeast to hyphal transition in Candida albicans, (2013).
[22] B. Lok, M.A.A. Adam, L.Z.M. Kamal, N.A. Chukwudi, R. Sandai, D. Sandai, The assimilation of different carbon sources in Candida albicans: Fitness and pathogenicity, Med. Mycol. 59 (2021) 115–125.
[23] X. Huang, X. Chen, Y. He, X. Yu, S. Li, N. Gao, L. Niu, Y. Mao, Y. Wang, X. Wu, Mitochondrial complex I bridges a connection between regulation of carbon flexibility and gastrointestinal commensalism in the human fungal pathogen Candida albicans, PLoS Pathog. 13 (2017) e1006414.
[24] R.E. Sandstrom, L. Stockman, Germ tube-positive Candida tropicalis, Am. J. Clin. Pathol. 69 (1978) 365.