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DIAGNOSIS AND STUDY OF YEAST USED IN TRADITIONAL MEDICINE AND THE EFFECT OF SERIAL MUTATION ON THEIR VITALITY

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Abstract

The present study includes identified isolate is used as a medication in traditional depending on morphological, cultural characteristics and biochemical tests. Moreover, their identification was confirmed by API 20 C (Analytic Profile Index) test. The results revealed that isolate belonged to the yeast *Candida colliculosa*. The results of combination effects of MNNG and UV study revealed that the killing intensity increased after mutagenesis with 0.2 mg/ml of MNNG and increasing UV period to 20 min, which reached to 100%.The ability of the strain *C. colliculosa* for resistance of 20 antibiotics. Results showed that the studied strain was resistant to Amoxicillin, Ampicillin, Candizole and Cephalexin monohydrate, Clindamycin, Erythromycin, Fluconazole, Gentamicin, Fluconazole, Lamisil, Neomycin, Penicillin, Rifampin, Streptomycin, Tetracycline, Trimethoprim and Vancomycin while it showed sensitivity to Chloramphenicol, Clotrimazol, Ketoconazole and Nystatin. After mutation with MNNG and UV, results revealed that the strain was resistant to Amoxicillin, Ampicillin, Candizole**,** Cephalexin monohydrate, Clindamycin, Erythromycin**,** Gentamicin**,** Neomycin, Penicillin, Rifampin, Streptomycin, Tetracycline, Trimethoprim.

Key words : Yeast, traditional medicine, mutation, vitality.

Introduction

Yeasts received increased attention by scientists for their effective role in many biological fields and variety, it is not limited to food fields only, as they were used In adding taste and flavor to fermented food and drinks (Hou *et al*., 2012), moreover, a number of yeasts occupy an important place in the production of enzymes such as lipase (Paludo *et. al.,* 2018) and alcohol (Modi *et al*., 2018). At the present time it has become Its killer effect against bacteria and fungi is widely known and this feature came based on their competitiveness on food and production of antimicrobial compounds such as Mycocins and Antibacterial Compounds (Hatoum *et al.,* 2012). It produces compounds of medicinal benefit such as insulin, viral hepatitis vaccine and HPV vaccine (Hou *et al*., 2012). As colored yeasts produced carotenoids pigments colorful anti-oxidant (Malla Obaeda, 2017). Yeasts are a source of some vitamins and drugs such as Vitamin A, D, B2, B6 and B12 (Zmudzinski, 2010) mannan sugar that derived from *S. cerevisiae*. (Al- Eqabi, 2009) entering in the manufacture of many medicinal and pharmaceutical materials that are used to treat various diseases directly or mixed with other drugs for example use in the treatment of cancer tumors, as well as to control diabetes and treat wounds and burns (Ragini *et al*., 2002).

R. glutinis was used in the biotransformation processes of some substances to obtain substances of interest in the pharmaceutical industry (Fardelone *et al*., 2011). Recently it has been observed that these microorganisms have great ability for developing the antibiotic-resistant characteristic (Milanezi *et al*., 2019) this is due to its own group of mechanisms resistance it may be resulting from the inhibition of Ergosterol synthesis which enters in formation of cell membrane which leads to formation holes that increase permeability of important materials to outside of the cell (Wiederhold, 2017) or mutation in the genes encoding transfers enzymes that transfer the drug inside the cell or change in target protein leads to increase its production and reduce of the toxic for the drug, thus the cell became resistance status (Hokken *et al*., 2019) this requires the development of

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new strategies treatment returned on biological knowledge of antibiotics and resistance to antibiotics (Usher and Haynes, 2019) so scientists interested to study and understand the mechanisms of their antibiotic resistance in detail. Strains of microorganisms, especially industrial ones, can be improved by causing a change in their genetic material while they are inside their cells. This is known as Mutation Induction. These changes can be at the level of the chromosome or at the gene level (Zubay, 1993), which may result from it. A change in traits such as an inability to synthesize some amino acids or vitamins, or an allergy or resistance to some antibiotics, etc. (Bruce *et al.*, 2002).

The aim of this study is to diagnose yeast used in folk medicine, test its resistance to antibiotics and study the effect of parasitism on its vitality and resistance to antibiotics.

Materials and Methods

Source Isolation

Isolate yeast from Nimrod area in Mosul city in the form of gelatins cuts in the yeast cells in a pot containing water and a superrey with a small amount of tea. A small piece of a yeast cut was wounded with a little liquid by the homogenizer. Publish a part of this suspended on the surface of Yeast Extract Malt Extract Agar Medium (YM Agar) and brushes with glass rod, The dishes were incubated in 28°C for 48 hours for individual colonies.

Identification Tests

Morphological characters of colonies and Microscopic examination.

The isolation planted in a Streaking on Malt Extract Medium (MEA) and incubated in 28°C for 28 hours, Recorded notes that related to the same characteristics and examined under the microscope at 40X to note the shape of cells (Pitt and Hocking, 2009).

Diazonium Blue B (DBB) Color Test

The yeast grown on YM Agar medium and incubated in 28°C for 10 days until the acus is formed in Ascomycetes. Added a drop to two drops from Diazonium Blue B reagent on the surface of the developing colonies and left to 2-3 minutes at laboratory temperature. The coloration of the colonies indicates that the dark red color, which is oblique to the violet, indicates that it belongs to the Basidiomycetes, but if it coloration orange, it indicates that it belongs to Ascomycetes (Kurtzman and Fell, 1998).

Growth in 25°C and 37°C Test

The ability of the isolation has been tested on the growth of 25°C and 37°C temperatures in MEA medium by Streaking, incubated in those termal degrees for 3-7 days.

Assessing Ability to Utilize Nitrate as a sole Nitrogen Source

The test was conducted by transfer the part of culture by Streaking on the surface of Czapek Agar medium in petri dishes. Incubated in 28°C for 3-7 days.

Assessing Preservative Resistance of Glacial Acetic Acid

Inoculate petri dishes container Malt Acetic Acid (MAA) solidified with part of culture by Streaking and incubated in 28°C for 3-7 days.

Growth at Reduced Water Activities in High Carbohydrate Levels

Inoculate petri dishes container MY50G medium with part of culture by Streaking and incubated in 28°C for 3-7 days.

Growth at Reduced Water Activities and High Level of Sodium Chloride

Planting isolation by streaking on MY10-12 medium incubated in 28°C for 3-7 days (Pitt and Hocking, 2009).

Mycelium Formation Test

Conducted the test to know the ability of yeast on forming true mycelium and pseudo mycelium, By inoculating small flasks container 20 ml from Sabouraud's Glucose Broth Medium (SGB) with part of pure culture from yeast, incubated the flask for 48 hours in 28°C then examined the yeast growth under microscope at 40X, budding, cell shape and presence of mycelium and its shape whether true mycelium or pseudo mycelium (Kurtzman and Fell, 1998).

Test System Analytic Profile Index (API 20C)

Tested according to Biomerieux company protocol by taking a new colony from yeast isolation growing on SGA medium after two days of incubation and transferred to a container tube 2 ml NaCl with concentration 0.85%. Shook the tube and pull them 100µl and adding a second tube container on a 7 ml medium (Equipped with kit by the fitted company) Shook the tube well then the special well in stripe was filled with yeast suspension. Incubated the strips for 48-72 hours in 29±2°C. Results recorded after 48 hours by naked eye through turbidity in pits, which produce from metabolism, the accuracy of these results was verified after 72 hours. Give the results of this test serial number seven, each digital group contains three numbers (1, 2 and 4) they are recorded in a special card book, The sequence of numbers is compared to the API manual, Determining the genus and species of yeast.

Effect of Serial Mutation Using Chemical Mutagen N-methyl-Ñ-nitro-Ñ-nitrosoguan-idine (MNNG) and Physical Mutagen UV.

Li *et al*., (2007) method was used in combination of chemical and physical precursors. 5ml of MNNG was added at a concentration of 0.2 mg/ml to a test tube containing 1ml of the yeast suspension. The tube is incubated in a water bath vibrating at 37°C for 30 minutes. Deposition of cells by centrifugation for 15 minutes at speed 900 cycle/ min, Repeat the washing process three times and suspend the precipitate by adding 10 ml sterile distilled water, shook tube well. The irradiation process was done inside a dark room and then took 5 ml of suspense placed in petri dish and place the dish on a magnetic stirrer work to move the suspension after lifting the cover circular movement during irradiation to ensure that all cells exposed to radiation. The irradiation process was done five times (2, 5, 10, 15 and 20) minutes, As well as zero treatment (without irradiation) comparison. After the irradiation period is over, pull the dish, wrap it with aluminum foil and leave in the dark for an hour to avoid photoreactivation. Then mix the suspension well and inoculation five dishes SGA medium with 0.2 ml of yeast suspension for each dish, The dishes were incubated in 28°C for 7 days then count the number of colonies that have grown in the five dishes to be the total number of colonies surviving in 1 ml of irradiated yeast suspension and for the time period studied. Attended from non-irradiated yeast (zero treatment) serial of dilution until 10^{-4} from this dilution, 10 dishes SGA were inoculated with 0.1 ml for each dish. The dishes were incubated in 28°C for 7 days then count the number of colonies that have grown the percentage of survivors and the percentage of death.

$$\text{Of Survivors} = \frac{\text{The resulting number of irradiation treatment}}{\text{The resulting number of non- irradiated}} \times 100\ \%$$

% of Kill = 100 - percentage of survivors

Table 1: Effect of the chemical mutagene MNNG and physical mutagene UV radiation on vitality.

Yeast	Period of extension to UV (minutes)	Average	(%) Survivors	(%) Kill
C. colliculosa	0 (cont.)	25.2	-	-
	2	18	71.42	28.58
	5	10	59.70	40.29
	10	5.2	39.68	60.32
	15	3	11.90	88.1
	20	0	0	100

Antibiotic Resistance Test of Wild Type and Mutant Yeast Isolate

For determining the resistance to the isolation of non-mutagenic and mutagenic yeast by combining MNNG chemical mutagen and ultraviolet radiation. Prepared yeast Extract Peptone Glucose Agar (YPG) medium with antibiotics at their final concentrations (Table 1). The yeast isolate inoculated with streaking method then incubated at 28°C for 28 hours and the results observed (Ernst and Chan, 1985).

Results and Discussion

Identification Yeast

• Culture Characteristics: The results showed that the appearance characteristics of yeast colonies grown on MEA medium that color is creamy, circular shape, diameter colonies 2 mm, with complete soft edges, a little convex, dark butter and soft (Fig. 1A), The initial diagnostic specification matching for mentioned (Kurtzman and Fell, 1998; Deák, 2008; Pitt and Hocking, 2009).

• Microscopic Examination: The results of the microscopic examination of yeast were shown to be budding, interminable ovoid (Fig. 1B). This result is consistent with the documented specification by Kurtzman and Fell, (1998).

• Mycelium Formation Ability Test: The results showed the yeast non-ability to mycelium formation.

Biochemical Tests

• Growth in 25°C and 37°C Test: The results of this test revealed the capacity of yeast on growth in 25°C and 37°C.

• Assessing Ability to Utilize Nitrate as a Sole Nitrogen Source: Observed not ability the yeast to utilization nitrate as a Single source of nitrogen, it was unable to grow and attribute the ability of yeast to tolerance or resistance to capacity on utilization of nitrate (Pitt and Hocking, 2009).

• Assessing Preservative Resistance of Glacial Acetic Acid: The results of this test showed that the isolate was positive to this test and this agreed with what Pitt and Hocking, (2009) has been report.

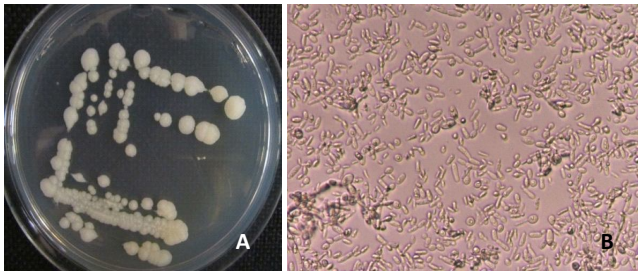

Fig. 1: Culture Characteristics and Microscopic of C. colliculosa.

Table 2: Resistance and sensitive of yeast for antibiotics.

Yeast	Antibiotics																			
	AX	AP	Cd	Cf	Cm	Clim	Ct	Er	Fcz	Gm	Kc	Ls	Nm	Nys	Pen	Rif	Str	Tc	Tm	Van
C. colliculosa	R*	R	R	R	S	R	S	R	R	R	S	R	R	S	R	R	R	R	R	R

* R=Refer to the resistance, S=Refer to the sensitive.

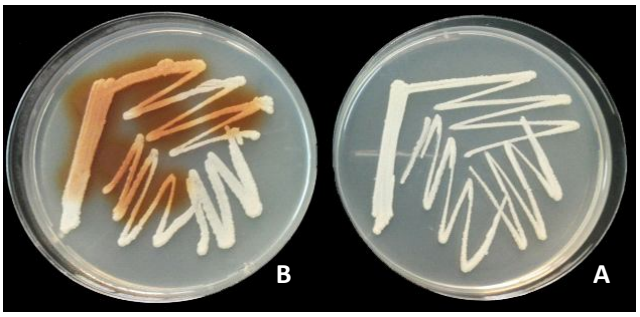

Fig. 2: Diazonium Blue B Color test to *C. colliculosa* that grow on YM agar for 10 days.

• Growth at Reduced Water Activities in High Carbohydrate Levels: The current results revealed that isolate was negative for the test and this agreed with (Pitt and Hocking, 2009).

• Growth at Reduced Water Activities and High Level of Sodium Chloride: The results showed that yeast was negative for the test and this result was according to Pitt and Hocking, (2009).

• Diazonium Blue B (DBB) Color Test: It was noted that the isolation was negative for the test as shown in (Fig. 2). This adjective is compatible with described from Kurtzman and Fell, (1998) and Malla Obaeda, (2017). This Ascomycetes and Basidomycetes.

• Testing the Biochemical Confirmation by API 2^0C Testing: API 2^0C was used to document and confirmation all pervious tests in determination genus and species in this study. The result obtained was shown during color and chemical changes of the fine test tubes was shown according to the manufacture's method of operation that isolation is *C. colliculosa* (Fig. 3). These results according with results has been obtained in a study (Park *et al*., 2019; Dewaele *et al*., 2019 and Arastehfar *et al.,* 2019).

Study Susceptibility Chemical (MNNG) and Physical Mutagene on the Vitality of Yeast

The interference with chemical and physical mutagens led to increased homicide rates for mutagenic isolation. They were very sensitive to the two mutagens in terms of non-growth with increase the exposure period to 20 minutes (Table 1). This is due to the strong influence of chemical and physical mutagens when combined with each other Sajdi and Ali, (1987) pointed out to concentrate of mutagenic substance and length of exposure time to rays, prolonged exposure for long period or used high concentration from mutagen substance led to the killing of cells exposed and this is according with a study Malla Obaeda, (2017).

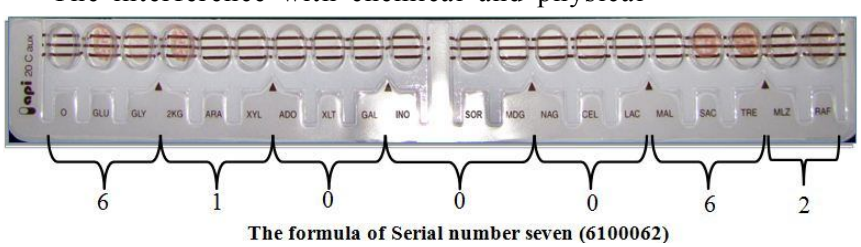

Fig. 3: API 20C Test to *C. colliculosa*

Antibiotics Resistance of Isolated Strains

Yeast isolation for antibiotics was studied and accepted by yeast to determine the effect of mutation process. Use 20 antibiotics according to the streaking method and the results of this are shown in the table 2. The table note shows that the tested yeast showed resistance to most antibiotics used Amoxicillin, Ampicillin, Candizole, Cephalexin monohydrate, Clindamycin, Erythromycin, Fluconazole, Gentamicin, Fluconazole, Lamisil, Neomycin, Penicillin, Rifampin, Streptomycin, Tetracycline, Trimethoprim and Vancomycin while it showed sensitivity to Chloramphenicol, Clotrimazol, Ketoconazole and Nystatin.

Soofy, (2013) observed all isolates of *Saccharomyces* was resistances to Nystatin. Other study by Al-Taeei, (2013) note that isolates of *C. albican* were shown resistance to Fluconazole, Itraconazole, Ketoconazole and Terbinafine except one isolate that showed sensitivity to Fluconazole and Itraconazole while all isolates were sensitive to Nystatin except only one. Habib and Al-Saadi, (2015) found that isolates *Candida* spp. was sensitive to Nystatin and their growth inhibition by 87.8% of the isolates from the total isolates. During a study of Malla Obaeda *et al*., (2018) showing that isolates *Cyto minuta* BA78, *R. glutinis*, BA83, *R. graminis* BA1, *R. mucilaginosa* BA58, *R. mucilaginosa* BA75, *R. mucilaginosa* BA61 and *S. cervisiae* BA179 were resistant to Ampicillin, Cephalexin monohydrate, Clindamycin, Neomycin, Rifampin, Streptomycin and Trimethoprim and sensitive to Clotrimazol.

Antibiotic susceptibility test of mutated yeast

Table 3: Resistance and sensitivity of yeast mutagene by using the combination of MNNG and UV for antibiotics.

Yeast	Antibiotics																			
	AX	AP	Cd	Cf	Cm	Clim	Ct	Er	Fcz	Gm	Kc	Ls	Nm	Nys	Pen	Rif	Str	Tc	Tm	Van
C. colliculosa	R*	R	R	R	S	R	S	R	S	R	S	S	R	S	R	R	R	R	R	R

* R=Refer to the resistance, S=Refer to the sensitive.

It was observed from table 3, that the tested yeast was showed resistance to all antibiotics used Amoxicillin, Ampicillin, Cephalexin monohydrate, Clindamycin, Erythromycin, Gentamicin, Neomycin, Penicillin, Rifampin, Tetracycline and Vancomycin while becoming sensitivity to Candizole and Trimethoprim and retained sensitivity to Chloramphenicol, Clotrimazol, Ketoconazole and Nystatin and become sensitivity to Fluconazole and Lamisil.

The sensitivity of yeast mutant towards some of antibiotics compared with non-mutant yeast may return to change in the genetic structure of the parent yeast may return to change in the genetic structure of the parent yeast which may have occurred in the gene are portable on plasmid or chromosome in the form that make this yeast sensitive to these antibiotics (Nycek *et al.,* 2000) because of the short-wave ultraviolet radiation effect that characterizes its high virulence ability, they are absorbed by nitrogen bases of the DNA at 260 nm and lead to changes in the structure and sequencing of nitrogen bases, which is reflected in the structure of the genetic material (Al-Zubair *et al*., 1991) in addition to the chemical mutagene MNNG which is characterized by its ability to single mutation type of transition mutations and transversion mutations (Stepnaya and Kulaev, 2004). This reached by Mula Oboida, (2017) when he tested the effect of serial mutation by using chemical mutagen MNNG and physical mutagen UV on the yeasts *Cytobasidium minuta* BA78, *Rhodotorula glutinis* BA83, *R. graminis* BA1, *R. mucilaginosa* BA58, *R. mucilaginosa* BA75, *R. mucilaginosa* BA61 and *S. cerevisiae* BA179.

In the *ERG11* gene replacement mutations were found to give *C. albicans* resistance to Fluconazole causing reduce intimacy between the target enzyme and Fluconazole, in a study done by Li-Juan *et al*., (2010) on one of the isolates *C.albicans* that resistance to Fluconazole which has got replacement mutation in the *ERG11* gene found that isolate was sensitive to Itraconazole and Voriconazole.

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