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Isolation and Diagnosis of Different Local Isolates of *Saccharomyces Cerevisiae* using DNA Sequencing

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ABSTRACT

In this study, ten isolates of *Saccharomyces cerevisiae* were isolated from the fruit market of Mosul city. The Isolates were identified by morphological and cultural, and biochemical tests. The results showed that these isolates belong to the genus *Saccharomyces cerevisiae*. Performing specific PCR using primers (SC2, SC1), purified genomic DNA from local Isolates were showed eight bands at one size (1178) base pairs. The sequence of nitrogen bases for specific PCR products for DNA samples purified from three elected local isolates was determined. The results of the analysis using the DNA BLAST/NCBI program showed a similarity in the ratio (99, 98, 87) % among local isolation sequences and standard strains sequences of *Saccharomyces cerevisiae* recorded in the gene bank.

Keywords- *Saccharomyces cerevisiae*, specific PCR, DNA sequencing.

I. INTRODUCTION

Saccharomyces cerevisiae (Baker's yeast) are monocellular eukaryote organisms that reproduce by budding, belonging to fungi [1]. Their colonies are flat, soft, moist, shiny or pale, white or creamy, cell diameter 5-10 µm. All *Saccharomyces* were able grow in aerobic condition on glucose, maltose, and trehalose, but not grow on lactose and cellulose, generally their growth on other sugars uneven, but lactose and fructose were one of the best fermenting sugars, where act on analyzing sugar to feed on it and divided again as its ability to consume sugar varies depending on whether it is grown aerobically or not, this feature has been used to identify species instead of appearance characteristics [2]. Yeast is an important food source as containing a large number of proteins as well as many minerals such as zinc, chromium, iron, and fiber, Therefore it has been relied on for purposes other than food. The Baker's yeast belongs to the Ascomycetes Division, which is divided into several orders, including Saccharomycetales, known as the budding yeasts, which includes the family of Saccharomycetaceae, including the genus *Saccharomyces* [3]. This yeast is always used as a model in fungus research involving molecular genetics or DNA analysis [4]. DNA sequencing is known as the identification and order of nitrogen bases (Adenine, Guanine, Cytosine, and Thymine) in the DNA molecule, the first academic research conducted using laboratory methods and as a result of the development of methods of determining DNA sequences, this technique has become easier, faster and more accurate [5]. The goal of this study is to isolate and diagnoses local isolates of yeast *S. cerevisia* by molecular methods such as specific (PCR) and DNA sequencing technology.

II. MATERIALS AND METHODS

Samples collection:

We collected samples from fruits that show traces of mold on its pericarps as well as suffering from internal fermentation and drenched barley (bran) added to the flour in the Mosul city.

Isolated and Identification

1. Sabouraud Dextrose Agar Medium (SDA): 4 g dextrose, 1 g peptone, and 1.7g agar. The contents dissolved in 90 ml of distilled water and adjusted the pH at 5.6, then were sterilized by the autoclave. The sugar was dissolved in 10 ml of distilled water and sterilized by filtration, After the medium cooled to 55 ºC, sugar was added and poured into petri dishes [6].

2. Yeast Peptone Glucose agar Medium (YPG): 1 g yeast extract, 0.5 g peptone, 0.2 g glucose, and 1.2 g agar. These contents were dissolved except for glucose sugar in 90 ml of distilled water and adjusted pH to 7.2. The medium was sterilized by autoclave. then the glucose was dissolved in 10 ml of distilled water and added to the medium after being sterilized by filtration, Cool the medium to 50-55 ºC and pour into petri dishes [7].

Biochemical Testes

Sugars fermentation test

The fermentation medium were prepared from dissolved 4.5g yeast extract, 7.5g peptone in 860 ml of distilled water. 0.1g of Methyl red dye were dissolved in 100 ml of distilled water, take 40 ml of this dye and add to the rest of the contents, pour the mixture into a conical flask by (90 ml/flask), then were sterilized by the autoclave. 2 g of each sugar were dissolved in 10 ml of distilled water and added to the flask after sterilizing by filtration. The contents of each flask were poured into sterile test tubes. Nine different types of sugar were used (Maltose, Rhaminose, Glucose, Sucrose, Manose,

Arabinose, Lactose, Galactose, and Xylose). Developing colonies of 48 hours of incubation were transferred to the tubes and placed on a shaker incubator at 28ºC for 10-12 days. The samples were observed every day. [8]

The resistance and sensitivity of local yeast to antibiotics and heavy metal salts

Different types of antibiotics were used, are : Chloramphenicol (Cm), Ampicillin (Ap), Nystatin (Nys), Erythromycin (Er), Amoxicillin (Ax), Tetracycline (Tc), Streptomycin (Str), Flagyl (Fl), and 5 types of heavy metal salts are $HgCl_2$, $CdCl_2$, $ZnCl_2$, $NiCl_2$, and $CoCl_2$ to determine the resistance and sensitivity of isolates to antibiotics and heavy metal salts, after the medium (YPG) cooled to 55-60 ºC, antibiotics and sterile heavy metal salts were added to medium both separately after sterilized by filtration at concentrations shown in table (1). The samples were cultured in a streaking method and were incubated for 48 hours at 28 ºC, the results were recorded. [9].

Table 1: Stock and final concentrations of antibiotics and heavy metal salts

Antibiotic and Heavy Metal Salts	Symbol	Stock Solution mg/ml	Final Concentration µg/ml	Solvent
Chloramphenicol	Cm	5	100	Absolute alcohol Ethanol
Ampicillin	Ap	20	100	Ethanol 70%
Nystatin	Nys	5	50	DMSO
Erythromycin	Er	10	100	Absolute alcohol Ethanol
Amoxicillin	Ax	5	100	distilled water
Tetracycline	Tc	10	100	Ethanol 50%
Streptomycin	Str	20	100	distilled water
Flagyl	Fla	5	50	distilled water
Mercuric Chloride	$HgCl_2$	50	100	distilled water
Cadmium Chloride	$CdCl_2$	50	100	distilled water
Zinc Chloride	$ZnCl_2$	50	100	distilled water
Nickel Chloride	$NiCl_2$	50	100	distilled water
Cobalt Chloride	$CoCl_2$	100	100	distilled water

Molecular Tests:

Extraction of genomic DNA

Genomic DNA was extracted from local isolates of yeast *S. cerevisiae*, using (Presto ™ Mini Genomic DNA Yeast Kit) (UKAS).

Electrophoreses

The method Kado and Liu, [10] was adopted in the preparation of agarose gel and its electrophoreses when 1.05g of agarose dissolved in 150 ml of the buffer solution 1X TBE at concentration 0.7%. Genomic DNA samples were carried in the agarose gel, a voltage difference (45 volts) were used for 15 minutes after which the voltages were increased to (70 volts) for 3 hours. Ultraviolet radiation at a wavelength of 254 nm exposed by the UV Transilluminator device to detect genomic DNA bands in the samples under test.

Specific Polymerase Chine Reaction (PCR)

This interaction were conducted in the DNA Lab (Scientific Research Center) of Mosul city using general primers forward SC1 (5′-AAC GGT GAG AGA TTT CTG TGC-3′) and reverse SC2 (5′-AGC TGG CAG TAT TCC CAC AG-3′) [11]. Through small tubes with a capacity of 0.2 ml. each reaction tube contains 10 µl of PCR Master Mix (2X), 1µl at concentrations (10 pmol/ µl) of each primer, 4 µl with a concentration of (100 ng) of DNA and were completed volume to 20 µl by sterile distilled water. The tubes were placed in a thermocycler using following program:

The initial denaturation at 95ºC for 6 minutes, followed by 35 cycles of the denaturation at 95ºC for 45 seconds, the annealing at 54º C for one minute, the elongation stage at 72ºC for one minute, the final elongation at 72ºC for 5 minutes. The samples were loaded into agarose gel at 2%. The results were detected using UV Transilluminator. [12].

Determination of the Nitrogenous Bases Sequence for Specific PCR Products

The sequence of nitrogen bases for specific PCR products for DNA samples purified from three elected local isolates (SY2, SY4, SY7) were determined using (Automatic Sequencer Applied Bio System Genitive Analyzer 3130, USA) and by Big Dyer Terminator V3.1 Cycle Sequencing Kit, in the Genome Center / College of Science and Health / Koya University / Kurdistan Region / Iraq.

Analysis of the Nitrogen Bases Sequence for Specific PCR Products and Determination of the Phylogenetic Tree

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The nitrogen bases sequence analysis of specific PCR products were performed using the DNA BLAST STAR/Laser website, the sequences of each sample were compiled and all sequences were edited using optical sampling. Sample sequences were entered on the NCBI BLAST website, which sends its to genome research in the gene bank via the electronic link: http://blast.ncbi.nIm.nih.gov/Blast.cgi

To identify existing sequences recorded within the Gene Bank and similar to the sequences under study. To classify the sequences of the samples under study and discover the relationship between them and other sequences, mole blast tools were used to determine which of these sequences are the closest and most relevant in terms of classification via the electronic link: http://blast.ncbi.nIm.nih.gov/moleblast/moleblast.cgi

III. RESULTS AND DISCUSSION

Diagnosis of Local Isolates

Ten local isolates of *S. cerevisiae* which obtained from fruit and vegetables were diagnosed based on the phenotype of colonies. The colonies were different colors between white and cream with smooth edges and smooth surfaces. The colonies diameter ranges between 2-8 mm, and its distinctive scent is an important characteristic in the phenotype diagnosis by observing the developing colonies on the medium of YPG 48 hours age, Figure (1). This result were agree with researchers [13] in terms of the colonies forms and the contrast of colors when they isolated 17 yeasts type from different types of fruits, also agree with results of [14] when were isolated 166 isolates belonging to different of yeasts obtained from apples and citrus fruits.

Figure (1) Morphology of local isolates farms for *S. cerevisiae* yeast

Microscopic Diagnosis

Local yeast samples were examined using a photomicroscope with magnification (10, 40, 100X) , were observed single, spherical or oval-shaped cells, it could see bud cells, Fig. (2), this result agreed with the description of researchers [12] regarding the shape of single cells in addition to being gram stain-positive.

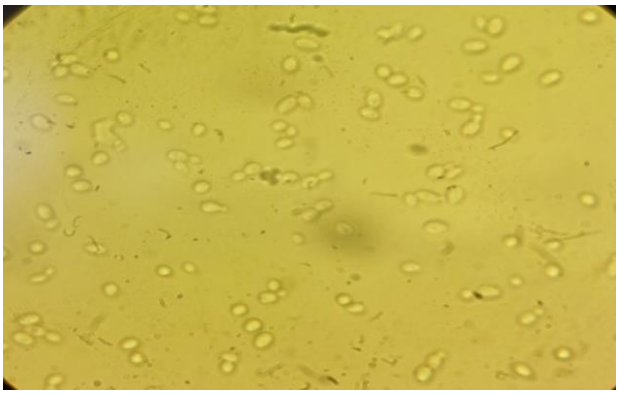

Figure (2) the local isolates cells of Saccharomyces cerevisiae under the microscope (100X).

Biochemical tests :

After obtaining the local isolates of the yeast and diagnosing it in terms of microscopic examination and the morphological of the colonies, the biochemical tests were carried out:

Sugars fermentation tests

The results showed ability that all isolates to ferment sugars (glucose, sucrose, maltose, mannose, and galactose) in the ratio 100% where the medium color changed from red to yellow to indicating the fermentation of sugar while were unable all isolates to ferment lactose sugar in the ratio 100%. The inability of the isolates to ferment lactose sugar is a characteristic of yeast S. cerevisiae because it is unable to produce B-galactosidase, which breaks down lactose into glucose and galactose so that yeast consume it as a carbonic source [15,16] while the sugar of xylose, rhamnose, and arabinose was able to ferment in the ratio (60, 60, 80%) respectively. table (2). These results are consistent with findings of Mpofu, *et al* (2008) [17] when he collected 16 isolates of S. cerevisiae and found that most of the isolates were able to ferment (glucose, galactose, sucrose, and rhamnose) and unable to ferment lactose.

Table (2) ability of isolates to ferment different types of sugar

Samples	Maltose	Rhamnose	Lactose	Sucrose	Manos	Arabinose	Galactose	Glucose	Xylose
SY1	+	-	-	+	+	-	+	+	-
SY2	+	-	-	+	+	+	+	+	-
SY3	+	-	-	+	+	+	+	+	-
SY4	+	+	-	+	+	-	+	+	-
SY5	+	+	-	+	+	+	+	+	+

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SY6	+	+	-	+	+	+	+	+	+
SY7	+	+	-	+	+	+	+	+	+
SY8	+	+	-	+	+	+	+	+	+
SY9	+	+	-	+	+	+	+	+	+
SY10	+	-	-	+	+	+	+	+	+

(+) ferment sugar, (-) not ferment sugar

Resistance and sensitivity of local isolates yeast S. cerevisiae to antibiotics and heavy metal salts

The results of table (3) showed the variation of local yeast isolates in their resistance to antibiotics, the isolates showed resistance to each chloramphenicol (Cm), ampicillin (Ap), tetracycline (Tc), streptomycin St in the ratio 100% while erythromycin (Er) and amoxicillin (Ax), most of the local isolates were resistant in the ratio (70, 90%). As for fungal antibiotic, the isolates showed sensitivity to nystatin (Nys) at 50 µg/ml with ratio 80% except (SY3, SY8), as well as Flagyl Fla where the sensitivity of local isolates was 90% at 50 µg/ml. the ability of nystatin to kill spores or inhabit their growth is due to its reaction with cell membrane causing damage to cells which leads to a change in selective permeability of cells. As for the effectiveness of the flagyl, it belongs to the possession of the nitro group of the metronidazole, which is chemically reduced by ferrodoxin and ferrodoxin-linked metabolic process, and the result of that process is responsible for the loss of helical structure of DNA resulting in inhibition DNA synthesis [18]. in a study conducted on yeast, It was observed, that nystatin at concentration 5-10 µg/ml acts as a fungicide of yeast *S. cerevisiae* while some types of local isolates were more resistant to nystatin at a concentration of more than 50 µg/ml [19]. In another study, were observed that yeast S. cerevisiae was very sensitive to both nystatin and flagyl at 50 µg/ml when isolating 16 samples, only one of which was resistant to flagyl and three samples to nystatin [20]. The results were showed from table (3) that all local isolates of *S. cerevisiae* were resistant to each of zinc chloride ZnCl2, nickel chloride NiCl2, and cobalt chloride CoCl2 in the ratio 100% as well as mercury chloride HgCl2 i.e all isolates have resistance except (SY2) that showed sensitivity. The sensitivity of the isolates was clear for cadmium chloride CdCl2 in the ratio 70%. The presence of cadmium or its salts in the yeast environment leads to the excitability of heat or hydrogen peroxide H2O2, and this response is very specialized as 32 genes are induced when treated with cadmium which contributes to the synthesis of amino acids containing sulfur necessary for the synthesis of glutathione enzyme. This enzyme controls the concentration of elements in the process of capture or detention [21] This result are correspond with results of researcher [22] which collected 52 isolates, all were resistant to zinc chloride and cobalt. This isolates varies in the extent of their resistance to lead chloride, 40 isolates were sensitive to cadmium chloride. While contradicting with results researcher [23] which indicated the ability of yeast S. cerevisiae to grow in the medium that content cadmium at concentration of 60 µg/ml after collecting 16 isolates, all isolates were able to grow in the medium except one.

Table (3) Resistance and sensitivity of local isolates *S. cerevisiae* yeast for antibiotics and heavy metal salts

Antibiotic and heavy metal salts	symbol	Final conc.	Samples understudy									
			SY1	SY2	SY3	SY4	SY5	SY6	SY7	SY8	SY9	SY10
Chloramphenicol	Cm	100	R	R	R	R	R	R	R	R	R	R
Ampicillin	Ap	100	R	R	R	R	R	R	R	R	R	R
Nystatin	Nys	50	S	S	R	S	S	S	S	R	S	S
Erythromycin	Er	100	R	R	R	S	S	S	R	R	R	R
Amoxicillin	Ax	100	R	R	R	S	R	R	R	R	R	R
Tetracycline	Tc	100	R	R	R	R	R	R	R	R	R	R
Streptomycin	Str	100	R	R	R	R	R	R	R	R	R	R
Flagyl	Fla	50	S	S	S	S	S	S	R	S	S	S
Mercuric Chloride	$HgCl_2$	100	R	S	R	R	R	R	R	R	R	R
Cadmium Chloride	$CdCl_2$	100	S	S	R	S	S	S	S	S	R	R
Zinc Chloride	$ZnCl_2$	100	R	R	R	R	R	R	R	R	R	R
Nickel Chloride	$NiCl_2$	100	R	R	R	R	R	R	R	R	R	R
Cobalt Chloride	$CoCl_2$	100	R	R	R	R	R	R	R	R	R	R

R= Resistant, S= Sensitive

IV. MOLECULAR DIAGNOSIS

Extraction of Genomic DNA

From figure (3) bands of DNA of equal size appeared because they traveled at distances of equal dimensions and with large sizes, due to the small distances they traveled in the agarose gel for all the purified genomic DNA samples of local yeast isolates.

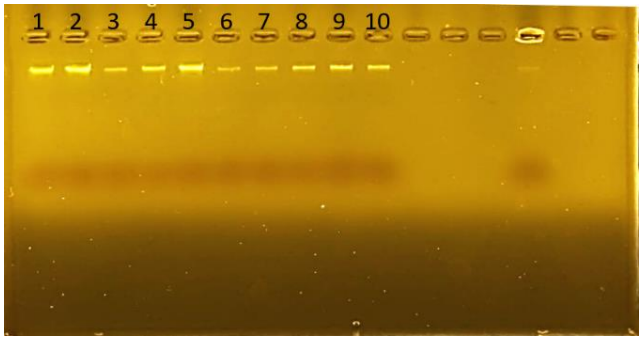

Figure (3) Results of Electrophoreses of genomic DNA content of yeast *S. cerevisiae* isolates through an agarose gel at 0.7%

PCR of Genomic DNA

The PCR was performed of DNA that purified from local isolate at Lab DNA center using primers: Forward SC1 (5′-AAC GGT GAG AGA TTT CTG TGC-3′) Reverse SC2 (5′-AGC TGG CAG TAT TCC CAC AG-3′)

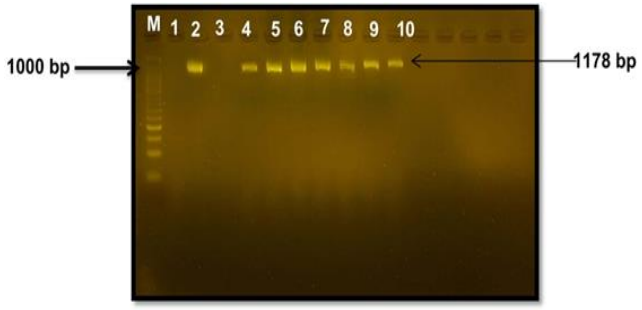

Figure (4) Results of a specific PCR of local yeast isolates, (1-10) represent of samples DNA have bands in one size (1178)bp, (M) represent Marker.

From figure (4), Purified DNA bands of local isolates can be observed of one size (1178 pb) which are the result of the specific PCR of purified DNA samples from local isolates (SY10, SY9, SY8, SY7, SY6 SY5, SY4, SY2), the appearance of these bands of DNA was due to the presence of common and similar sequences in the sequence of their nitrogen bases in the genomic DNA of these isolates, that able to complete those in the primers and the generation of DNA similar size bands. On the other hand, no band of DNA has shown as a result of a specific PCR of the two isolates (SY3, SY1) in the agarose gel, that the absence of DNA bands may be due to the failure of the primers binding to the DNA or the absence of sequences of nitrogen bases in the genomic DNA of these isolates that can be binding with the primer and make a reaction. These results are consistent with results of researchers [11] when tested 19 different isolates using the same primers SC1, SC2, which were obtained (1170 pb) size bands too. as such these results consistent with researchers [24] which were obtained 40 yeast isolates from the soil of different regions of Egypt, 4 of which were diagnosed for Saccharomyces cerevisiae by PCR technology using specific primers SC1 SC2, the bands were (1190 pb) size. These results are also agreeing with results [25], which collected 80 samples of yeasts from different sources, were diagnosed 15 isolates of which belong to *Saccharomyces cerevisiae* by PCR using specific primers (SC1, SC2) and the bands were (1170 pb) size. The purpose of the design of the primers SC1, SC2, to distinguish between Saccharomyces cerevisiae and Saccharomyces bayous where those primers proved the accuracy and speed of distinguishing among species, and we can be noted that the size of the bands ranges from (360-1290 pb) as the concentration of DNA changes from 1-10 micrograms in the PCR mixture, and that the decrease in the DNA quantity produces non-specific amplified of size. [11]

Determination of Nitrogen Bases Sequence for Specific PCR products

The specific polymerase chain reaction products of purified DNA samples were taken from 3 local isolates, which were selected from 8 isolates that gave a positive result in PCR reaction, namely isolates (SY2, SY4, SY7) and sent to the Genome Center / College of Science and Health / University of Koya, where sequences of nitrogen bases were obtained for DNA samples:

SY2

TTTCAACAACGGATCTCTTGGTTCTCGCATCGAT
GAAGAACGCAGCGAAATGCGATACGTAATGTG
AATTGCAGAATTCCGTGAATCATCGAATCTTTG
AACGCACATTGCGCCCCTTGGTATTCCAGGGGG
CATGCCTGTTTGAGAGTCATTTCCTTCTCAAACA
TTCTGTTTGGTAGTGAGTGATACTCTTTGGAGTT
AACTTGAAATTGCTGGCCTTTTCATTGGATGTTT
TTTTTTCCAANAGAGAGGTTTCTCTGCGTGCTTG
TTTTCATTGGATGTTTTTTTTCCAAA.

These sequences are entered into the DNA BLAST analysis program by link: - http://blast.ncbi.nlm.nih.gov/Blast.cgi.

To analyze these sequences and show their similarity with existing and recorded gene bank sequences. the results of the analysis showed similarity with ratio at 99% between these sequences and the standard isolation sequences of *Saccharomyces cerevisiae* Isolates OM20 recorded in the Gene Bank (MT136553.1). figure (5.)

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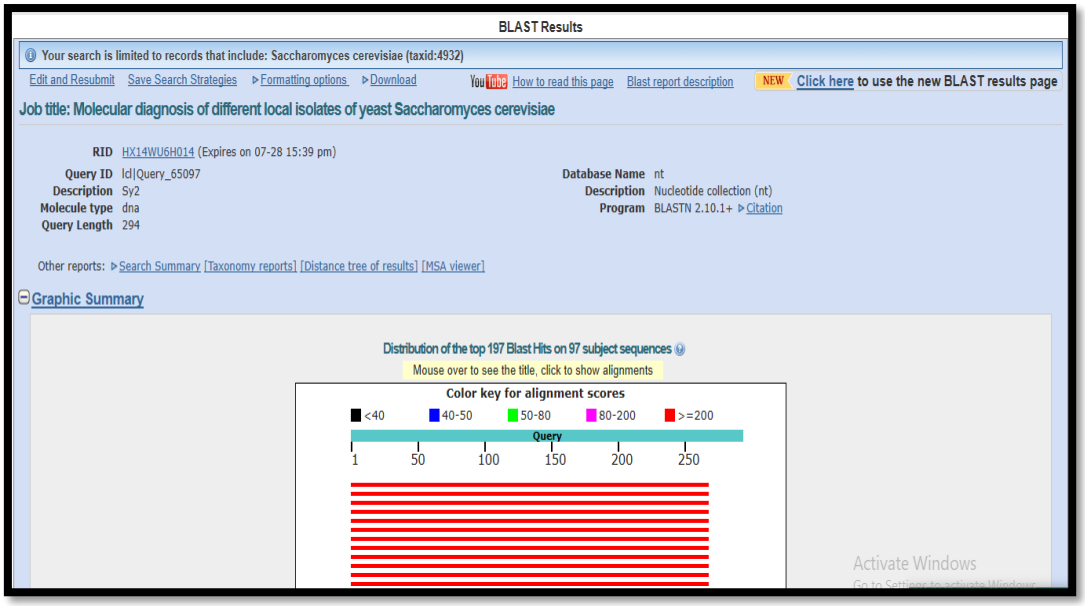

Figure (5) Analyses program of nitrogenous base sequences of local yeast isolate SY2, DNA Blast / NCBI .

Saccharomyces cerevisiae isolates OM20 Internal transcribed spacer 1, Partial sequence 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence, and large submit ribosomal RNA gene, partial sequence. Sequence ID: MT136553.1 Length:780 Number of Matches :1

Figure (6) Comparison of the nitrogenous base sequences between the local isolate SY2 and the standard strain MT136553.1

SY4
TTTGCAACTTTTTCTTTGGGCATTCGAGCAATCG
GGGCCCAGAGGTAACAAACACAAACAATTTTAT
CTATTCATTAAATTTTTGTCAAAAACAAGAATTT
TCGTAACTGGAAATTTTAAAAAATAAAAAACTT
TCAACAACGGATCTCTTGGTTCTCGCATCGATG
AAAAACGCAGCGAAATGCGATACGTAATGTGA
ATTGCAAAATTCCGTGAATCATCGAATCTTTGA
ACGCACATTGCCCCCCTTGGAATTCCAGGGGGC
ATGCCTGTTTGAGCGTCATTTCCTTCTCAAACAT
TCTGTTTGGTAGGGAGTGATACTCTTTGGAGTTA
ACTTGAAATTGCTGGCCTTTTCATTGGATGTTTT
TTTTCCAAA.

The results of the analysis using the DNA BLAST analysis program showed presence of similarity with ratio at 98% with sequences recorded in the gene bank at MK996251.1 Figure (7).

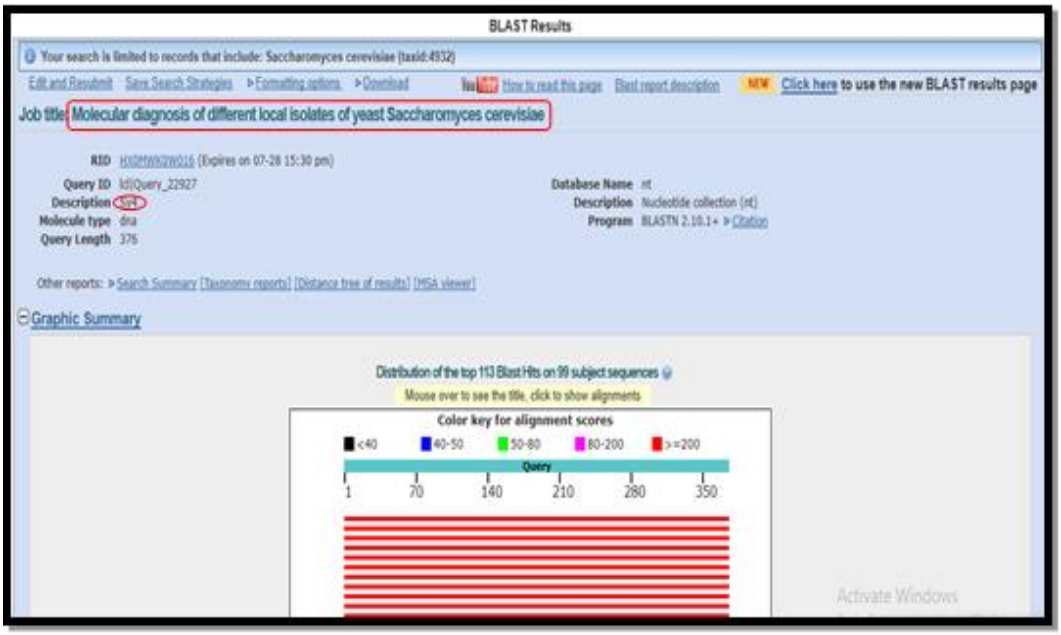

Figure (7)) Analyses program of nitrogenous base sequences of local yeast isolate SY4, DNA Blast / NCBI

Saccharomyces cerevisiae isolates MIBA783, Internal transcribed spacer 1, Partial sequence 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence, and large submit ribosomal RNA gene, partial sequence.Sequence ID: **MK996251.1** Length:617 Number of Matches :1

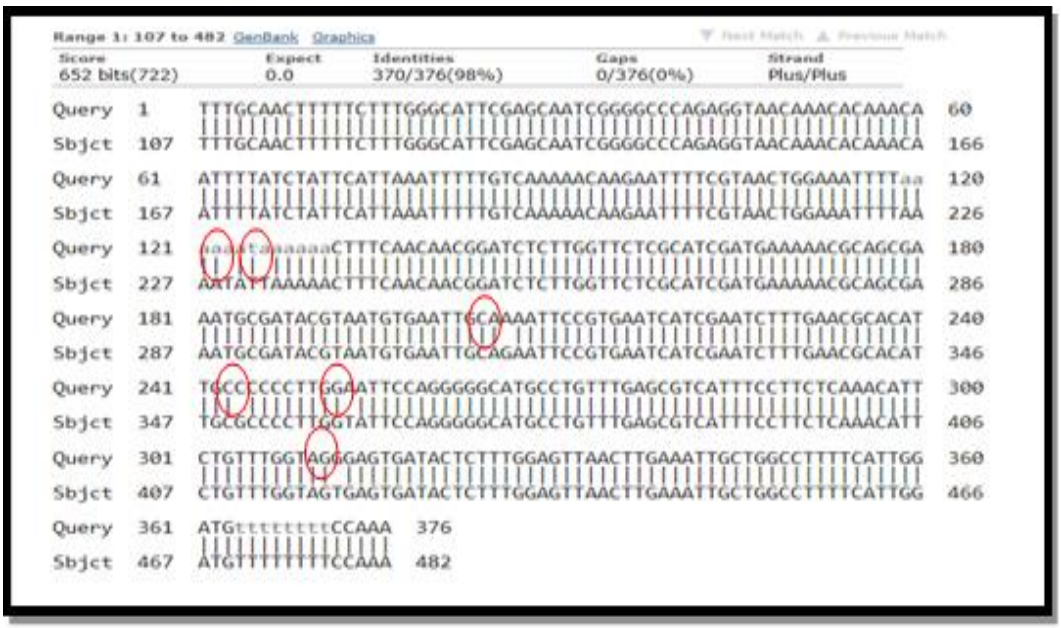

Figure (8) Comparison of the nitrogenous base sequences between the local isolate SY4 and the standard strain MK996251.1

SY7
GAGCGGAGGGCCAGAAACACCATGTGCGATCA
AATGCCCTTCCCTTTCAACAATTTCACGTACTTT
TTCACTCTCTTTTCAAAGTACTTTTCATCTTTCCA
TCACACTGTACTTGGGTGCTATCGCTCTCTCGAG
AAAACAACTTCGACTCTTCGAAGGGACTTTAGA
AATACCCGCACTCCTC. Also, the results showed presence similarity at 87% between SY7 isolation sequences and *saccharomyces cerevisiae* sequences, recorded in the gene bank at (KR063023.1) figure(9).

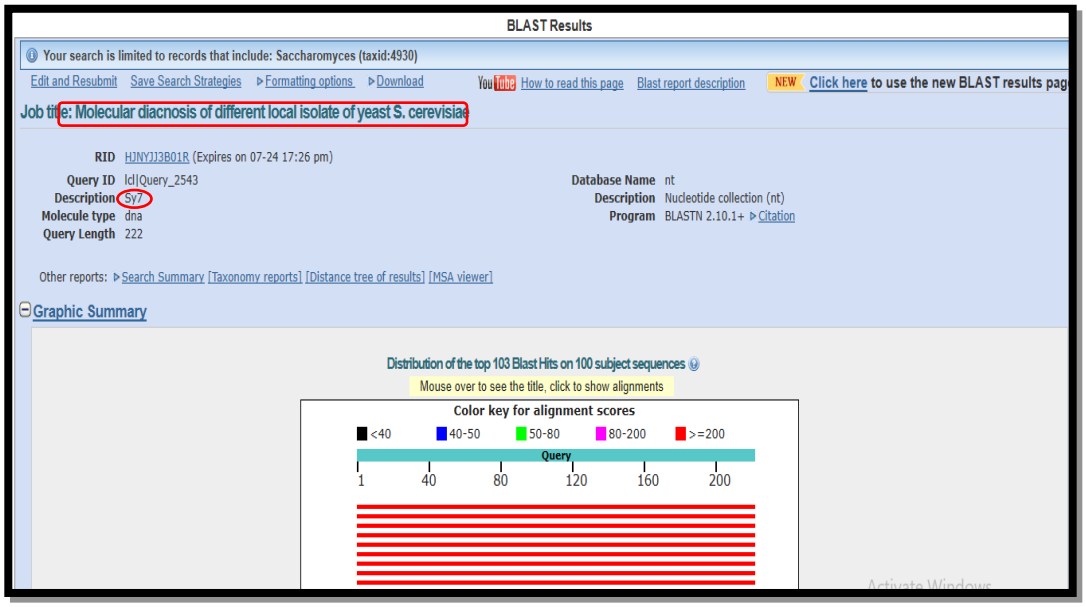

Figure (9) Analyses program of nitrogenous base sequences of local yeast isolate SY7, DNA Blast / NCBI .

Saccharomyces cerevisiae isolates B9 26S ribosomal RNA gene and internal transcribed spacer 1, ribosomal RNA gene, partial sequence. Sequence ID: KR063023.1 Length:554 Number of Matches :1

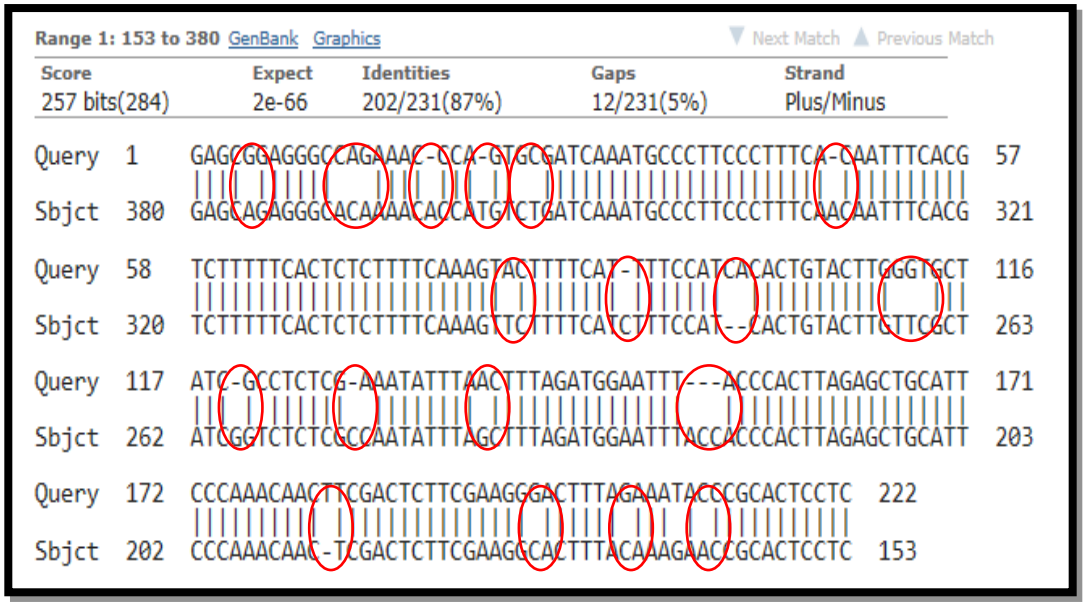

Figure (10) Comparison of the nitrogenous base sequences between the local isolate SY7 and the standard strain KR063023.1

From the results of the analysis It was appeared that there is a difference in some nitrogenous bases between the local isolates and the standard strains similar to them from the gene bank in some sites when comparing the sequences between the local and standard isolates, and this difference represents point mutation occurred at the level of DNA sequences of local isolates, which include replacing one base with another in the sequence of nitrogen bases or elimination a base from the sequence or adding a base to the sequence. Substitution mutations that have occurred are transition mutations, i.e. substitution involves replacing a nitrogen base with another of the purine or pyrimidine group themselves. and that these mutations have occurred automatically. Table(4).

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Table (4) Variation in the positions of nitrogenous bases between local isolates and similar standard strain from Gen Bank

The corresponding standard strain in the gene bank	Nitrogenous bases of the isolates under study and their corresponded		Nitrogenous base sequence site
	Local isolate	Standard strain	
S. cerevisiae **MT136553.1**	A	C	147
	A	--	245
S. cerevisiae **MK996251.1**	A	T	123
	A	T	126
	A	G	206
	C	G	244
	A	T	253
	G	T	312
S. cerevisiae KR063023.1	G	A	5
	C	A	12
	A	C	13
	G	A	14
	--	A	19
	--	T	23
	G	C	26
	C	T	27
	--	A	50
	A	T	82
	--	C	91
	C	--	99
	A	--	100
	G	T	112
	G	T	113
	T	C	114
	--	G	120
	--	C	129
	A	C	130
	A	G	139
	--	A	155
	--	C	156
	--	C	157
	T	--	182

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	G	C	198
	G	C	205
	C	A	211

The sequences of nitrogen bases which obtained from the DNA Sequencing technology were introduced into mole blast via the electronic link: http://blast.ncbi.nlm.nih.gov/moleblast.cgi.

A phylogenetic tree pattern were obtained to clarify the relationship and convergence between the local isolates of yeast under study and those of the strains recorded in the gene bank. figure (11).

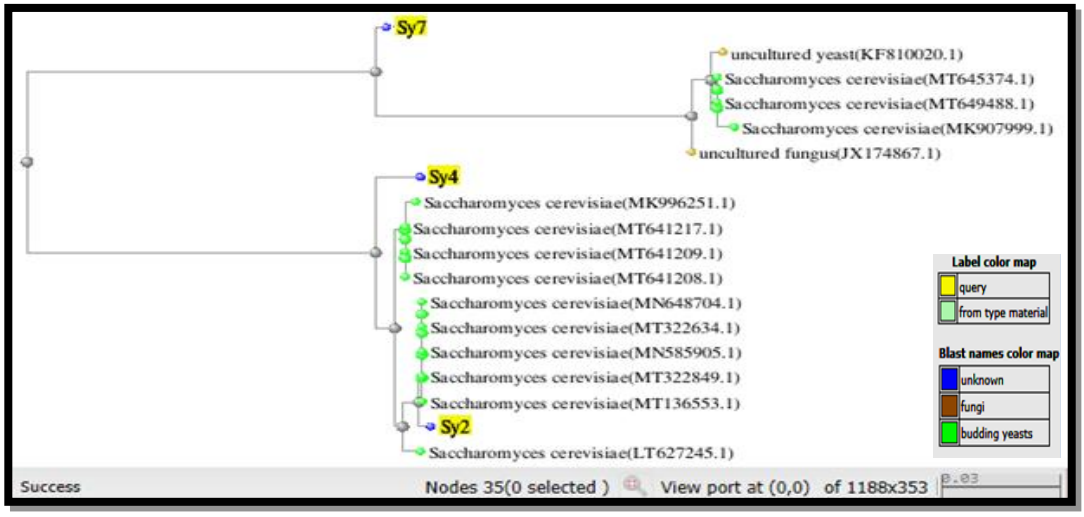

Figure (11) Phylogenetic tree of the results of nitrogenous base sequence analysis of three local isolates using Mole-Blast.

From the genetic Phylogenetic Tree it was observed that the local yeast isolate SY2 was close to the standard strain recorded in the Gene Bank S. cerevisiae MT136553.1 , *S. cerevisiae* MT322849.1 as well as close to *S. cerevisiae* MN 585905.1 and also close to *S. cerevisiae* LT627245.1

The local isolate SY4 has appeared closer to the standard strains *S. cerevisiae* MK966251.1 , *S. cerevisiae* MT641217.1, *S. cerevisiae* MT641209.1 as well as to local isolate SY7. It were observed that the local isolate SY7 was closer to the strains *S. cerevisiae* MT645374.1 , *S. cerevisiae* MT649488.1 and *S. cerevisiae* MK907999.1. These results were proved that the local isolates under study are belonged to yeast *S. cerevisiae*, which confirms and reinforces the results were obtained from the specific PCR technology and validates the diagnostic results were obtained in this study. These results are consistent with many research and studies. In study of [26], 61 isolation of yeast *Saccharomyces cerevisiae* were diagnosed using the technique of PCR by the specific primers SC1 and SC2 and to determine which strains are the most efficient in the production of ethanol using the technique of DNA sequencing and diagnosed many strains of them:- *Saccharomyces cerevisiae* PE-2, *Saccharomyces cerevisiae* JAY270, *Saccharomyces cerevisiae* RM11-1a, *Saccharomyces cerevisiae* YPS163, *Saccharomyces cerevisiae* YJM789, *Saccharomyces cerevisiae* AWRI1631. The results showed that the best-produced strains of alcohol are the *Saccharomyces cerevisiae* PE-2 strain followed by the *Saccharomyces cerevisiae* JAY270 strain. In one of the studies, the researcher mentioned the possibility of using DNA Sequencing Technique in the diagnosis of a group of proteins present in yeast Saccharomyces cerevisiae receives external signals and responds to different stimuli and then delivers them into the cell and that this diagnosis will enable scientists to control it in the future and use it to manufacture drugs contribute to the treatment of different diseases [27]. In other study included 155 samples, 20 of which were diagnosed as Saccharomyces cerevisiae, they noted that DNA sequence technology can contribute to identifying isolates and providing the necessary information to understand the process of fermentation in baking yeast. [28].

V. CONCLUSION

In this study, can be isolates yeast *saccharomyces cerevisiae* from the fermented fruits. Using genome sequencing can be diagnosis the yeast *Saccharomyces cerevisiae* at level species as an advanced method for diagnosing living organisms

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