

Isolation and Identification of some Yeasts from some Plants

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ABSTRACT

The present study included isolating 71 local isolates of yeasts during three months, starting from 1/10/2021 to 1/1/2022 from different plant sources, as the fruits were obtained from local markets in Mosul, Kirkuk, Erbil and Dohuk and from home gardens. The isolates were diagnosed after culturing on solid nutrient medium MEA (Malt Extract Agar Medium) based on phenotypic tests including colony color, shape, diameter, nature of its edges, height, texture, brightness, surface shape, culture and microscopy at 40X powers to observe the shape of yeast cells and measure their sizes Using (E 10X) ocular and biochemical including Diazonium Blue B (DBB) Color test, growth in 25°C and 37°C, assessing ability to utilize nitrate as a sole nitrogen source, assessing preservative resistance of glacial acetic acid, growth at reduced water activities in high carbohydrate levels, growth at reduced water activities and high level of sodium chloride and ability mycelium formation test. The results showed that it belongs to 17 different species of yeasts *Debaromyces*, *Rhodotorula*, *Pichia*, *Candida*, *Kluyveromyces*, *Geotrichum*, *Kloeckera*, *Saccharomyces*, *Cryptococcus*, *Zygosaccharomyces* and *Trichosporon*.

Keywords: Yeasts, Isolation, Diagnosis, morphology, Biochemical tests.

INTRODUCTION

Yeasts are a group of unicellular fungi belonging to ascomycota and basidiomycota, ranging in size from 3-10 μm in diameter and 5-30 μm in length (Maragatham and Panneerselvam, 2011). In recent years, interest in it has increased greatly due to the large number of evidence indicating its benefits and importance to human health. As it is a source for the production of ethanol production (Liu and Dien, 2022), biofuels and the production of compounds of medical benefit (Segal-Kischinevsky *et al.*, 2022) such as carotenoids, which are the raw material for vitamin A (Lobo *et al.*, 2021). and several fatty acids (Zhang *et al.*, 2022) and enzymes as pectinase (Malla Obaida, 2021).

Plants (the surfaces of fruits, flowers, leaves and grains) are common environments for the growth of yeasts, which grow on the surfaces of the leaves of plants and obtain their nourishment from the secretions of these leaves (Pollock, 1992). Several studies have indicated the possibility of isolating yeasts from fruits, flowers and leaves Suryaningsih *et al.* (2018) and Gaharwar *et al.* (2020) isolated the yeast *S. cerevisiae* from pineapple and Sapota fruits. Christita *et al.* (2021) was able to isolate yeast *Taphrina betulina* from the leaves of *Betula pendula* trees, which causes witches' broom disease. In a study conducted by Haedar *et al.* (2021), 12 isolates of yeasts were isolated from palm leaves belonging to different genera *Candida*, *Saccharomyces*, *Yarrowia*, *Brettanomyces*, *Endomycopsis*, *Rhodotorula*, *Rotula* and *Debaryomyces*. In the study of Hou *et al.* (2022) isolate yeasts *S. cerevisiae* and *Hanseniaspora vineae* from the fruits of apples, cantaloupe, grapes, guava, kiwi, mango, peach, passion fruit, dragon fruit or pitayas. The aim of the research is to isolate and identification the yeasts present in the local environment and to identify the environments from which yeasts required in many industries on which biotechnology depends.

MATERIALS AND METHODS

Collection of yeasts

71 yeast isolates were obtained from different plant sources, including fruits and leaves, which were obtained from local markets in Mosul, Kirkuk, Erbil and Dohuk, and from house gardens, starting from 1/10/2021 to 1/1/2022. Samples were randomly selected with different numbers for each species.

Isolation of yeasts

Yeasts were isolated from the fruits and leaves of different plants, according to (Obasi *et al.*, 2014; Zin *et al.*, 2021).

Identification tests

Morphological characters of colonies and microscopic examination

The isolates were grown in a streaking method on Malt Extract Medium (MEA) and incubated at 28°C for 48 hours. Observations related to morphological characteristics were recorded (Baidya *et al.*, 2016; Nasir *et al.*, 2017) and examined under a light microscope by using methylene blue dye at Powers 40X to observe the shape of yeast cells (Rahmana *et al.*, 2016; Citra, 2019).

Diazonium blue B (DBB) Color Test

This test was carried out according to the method of Kurtzman and Fell (1998).

Growth at 25° C and 37° C Test

Yeasts were cultured on solid media MEA by the method of streaking, and incubated at a temperature of 25 °C and 37 °C for a period of 3-7 days. The results recorded as negative when there is no growth or positive when there is growth (Pitt and Hocking, 2009).

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 (Pitt and Hocking, 2009).

Assessing preservative resistance of glacial acetic acid

Inoculate petri dishes container Malt Acetic Acid (MAA) solidified with part of culture of the studied isolates by streaking and incubated in 28°C for 3-7 days (Pitt and Hocking, 2009).

Growth at reduced water activities in high carbohydrate levels

Inoculate petri dishes containing solidified MY50G medium with part of each of the study isolates by Streaking and incubated in 28°C for 3-7 days (Pitt and Hocking, 2009).

Growth at reduced water activities and high level of sodium chloride

Inoculate 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 yeasts on forming true mycelium and Pseudomycelium, by inoculating small flasks containing 20 ml from Sabouraud's Glucose Broth Medium (SGB) with part of pure culture of yeasts, incubated the flask for 48 hours in 28°C then examining 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).

RESULTS AND DISCUSSION**Isolation**

The growth of colonies on the used media showed the presence of 71 isolates, as the highest number of isolates was 5 within the fruits of persimmons, while the lowest number was 1 from the fruits of each of bananas, annona, hawthorn, cranilla, tot, mancotine, guava, pears, figs and mangoes. While isolates from tree leaves showed the presence of 28 isolates, 4 isolates from grape leaves and apricot leaves, in addition to 1 isolate for each of the leaves of Japanese orange, orange, lemon, cinnabar, tangerine, apple and toth.

These isolation results were in agreement with many studies that indicate that yeasts are widely spread in nature, as they are found on the outer surface of fruits, leaves, and stems (Malla Obaeda, 2017; Segal-Kischinevzky *et al.*, 2022).

Table 1: The Sources of yeasts isolates of and its numbers.

	Isolate sources	Isolates number	Isolation symbol
The Fruits	khaki	5	M1, M2, M3, M4, M5
	Grape	3	M6, M 7, M8
	Sendi	3	M9, M10, M11
	Banana	1	M12
	Lebanese cream	1	M13
	Hawthorn	1	M14
	Avocado	3	M15, M16, M17
	Crandela	1	M18
	Tooth	1	M19
	Mancustine	1	M20
	Guava	1	M21
	Pomegranate	3	M22, M23, M24
	Pear	1	M25
	Coconut	2	M26, M27
	Lemon	2	M28, M29
	Tangerine	2	M30, M31
	Stump	2	M32
	Yellow Apple	2	M33
	Red Apple	2	M34
	Olive	2	M35
	Orange	2	M36, M37
	Figs	1	M38
	Mango	1	M39
The Leaves	Japanese Orange Leaves	1	M40
	Orange Leaves	1	M41
	Sour Lemon Leaves	1	M42
	Sindhi Leaves	1	M43
	Tangerine Leaves	2	M44, M45
	Citrus Aurantium Leaves	3	M46, M47, M48
	Apple Leaves	1	M49
	Encodenia Leaves	3	M50, M51, M52
	Grape Leaves	4	M53, M54, M55, M56
	Tooth Papers	1	M57
	Palm Leaves	2	M58, M59
	Pear Leaves	2	M60, M61
	Nabek Leaves	1	M62, M63, M64
	Apricot Leaves	4	M65, M66, M67, M68
	Pomegranate Leaves	1	M69, M70, M71
The Total		71	

Identification

Cultural Characteristics

The Morphological characteristics of colonies growing on MEA medium, in addition to microscopy as a preliminary identification, showed the presence of a number of isolates belonging to the same species, so 17 isolates were selected (Table 2). The initial diagnostic characteristics of these isolates were identical to what was mentioned (Kurtzman and Fell, 1998; Deák, 2008; Pitt and Hocking, 2009).

Table 2: Morphological characteristics of yeast colonies grown on MEA

Isolation	Morphological characteristics							
	Colony color	Colony shape	Colony diameter (mm)	Nature of colony edges	Colony height	Colony texture	Colony luster	Colony surface shape
M6	White	Circular	1.5	Irregular	Convex	Bowl	Opaque	Rough
M9	White	Circular	3.4	Smooth	Flat	Bowl	shiny	Soft-smooth
M12	off white	irregular	4	Irregular	Flat high center	Bowl	shiny	smooth
M13	creamy	Circular	2.5	Smooth	Flat	Bowl	shiny	Soft-smooth
M15	White	irregular	5	Irregular	Flat	Membranous-dermal	Opaque	Rough
M18	White	irregular	5	Irregular	Flat	chalky	Opaque	Rough
M21	White	irregular	11	ciliated	Flat high center	dermal	Opaque	Rough
M26	creamy	Circular	2.6	Smooth	High center	Bowl	shiny	Soft-smooth
M28	creamy	irregular	3.1	Irregular	Convex	Bowl	Opaque	Rough
M32	creamy	Circular	1.3	full soft	High	Bowl	shiny	Soft-smooth
M36	creamy	Circular	6.2	Smooth	Flat	Bowl	Opaque	Soft-smooth
M38	creamy	Circular	4.2	lobular	Flat	looks like cheese	Opaque	Rough
M41	White	Circular	2.7	Smooth	Flat	Bowl	shiny	Soft-smooth
M44	off white	Circular	8	Irregular	High center	Bowl	Opaque	Soft-smooth
M55	creamy	Circular	2.6	Smooth	Convex	Bowl	shiny	Soft-smooth
M59	Orange	Circular	1.9	full soft	Convex	mucous	shiny	Soft-smooth
M65	White	irregular	4.2	Smooth	High	Bowl	Opaque	Rough

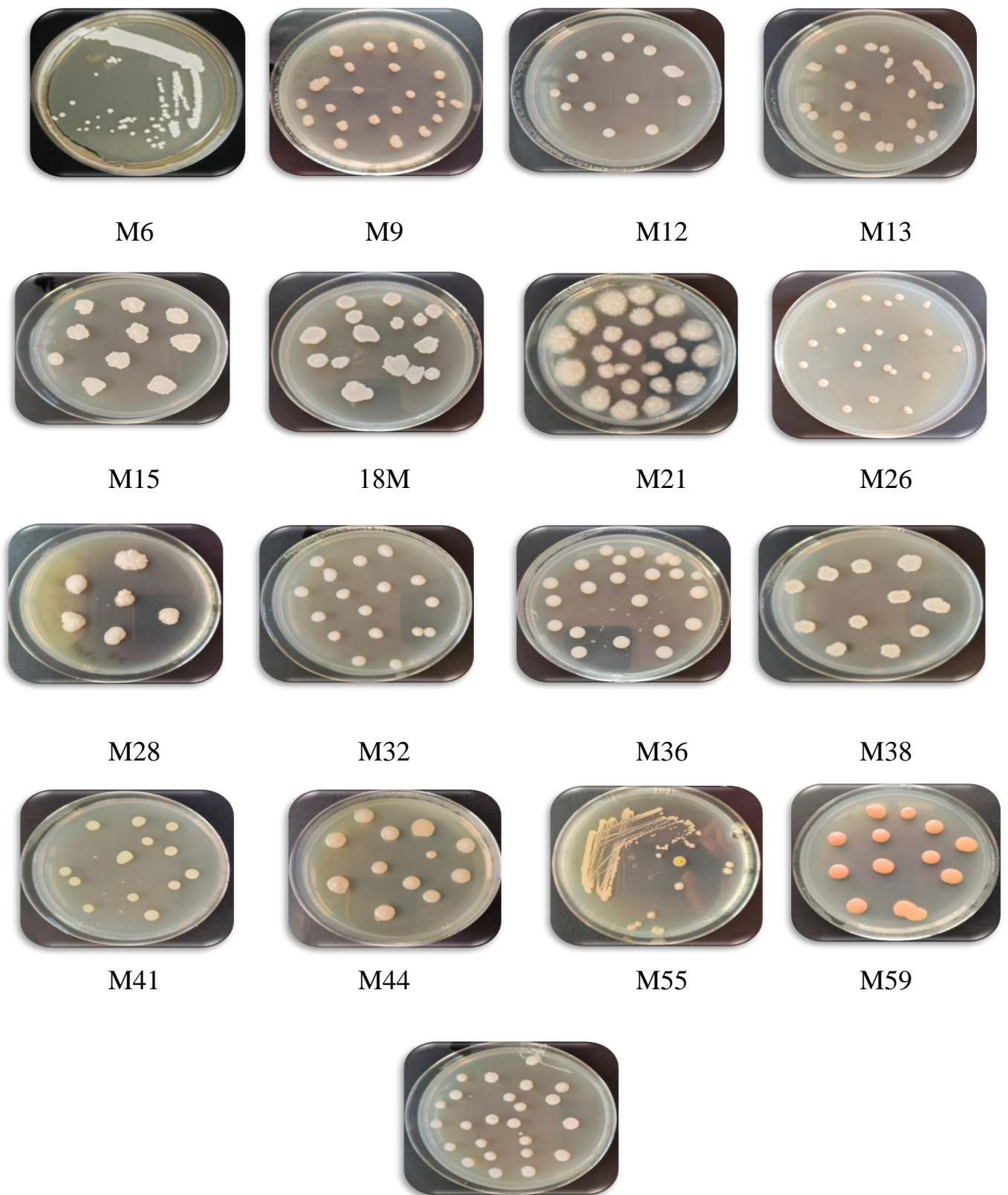


Fig. 1: Morphological characteristics of colonies of isolated yeasts grown on MEA at 48 hours old

Microscopic Examination

The results of microscopic examination of isolates showed a clear variation among them, whose cell shapes were budding, spherical, oval or elongated lemony, and some of them were elongated oval and of different sizes (Table 3). This is a characteristic of yeasts. From the foregoing phenotypic diagnosis of the developing colonies and examination of their microscopic smears, it was found that the isolates belong to the genera *Debaromyces*, *Rhodotorula*, *Pichia*, *Candida*, *Kluyveromyces*, *Geotrichum*, *Kloeckera*, *Saccharomyces*, *Cryptococcus*, *Zygosaccharomyces* and *Trichosporon*. (Kurtzman and Fell, 1998; Deák, 2008; Pitt and Hocking, 2009).

Mycelium Formation Ability Test

The results of current study showed the ability of 9 (52.94%) isolates to give pseudo-mycelium, while the results showed the ability of seven isolates (*Candida* M9, *Candida* spp. M13, *Geotrichum* spp. M18, *Candida* spp. M21, *Candida* spp. M36, *Trichosporon* spp. M38 and *Cryptococcus* spp. M44) with a percentage of 41.17% on the formation of true mycelium, and only one isolate *Rhodotorula* spp. M59 5.88% with its ability to give mycelium, some of it being true mycelium and some of it being false mycelium Fig. (2) . This characteristic is considered to be variable in these types of yeasts and these results are in agreement with Kurtzman and Fell (1998) and Malla Obaeda (2017).

Table 3: Microscopic examination of diagnosed yeasts isolates

Yeasts	Microscopic examination		
	Cell Shape	Cell Dimensions (µm)	Mycelium Formation Test
<i>Pichia</i> spp. M6	Spherical some are slightly elongated	3.5	–
<i>Candida</i> spp. M9	Ovoid	3.5*5	+
<i>Kluyveromyces</i> spp. M12	Ovoid	8*6	–
<i>Candida</i> spp. M13	Ovoid	7.6*14	+
<i>Candida</i> spp. M15	oval elongated	6.9*9	–
<i>Geotrichum</i> spp. M18	spherical	5	+
<i>Candida</i> spp. M21	Ovoid	7.7*12	+
<i>Saccharomyces</i> spp. M26	Spherical - small lemon	7	–
<i>Pichia</i> spp. M28	oval-cylindrical	6*4	–
<i>Zygosaccharomyces</i> spp. M32	Spherical slightly elongated	3.5	–
<i>Candida</i> spp. M36	Spherical slightly elongated	4.5	+
<i>Trichosporon</i> spp. M38	Ovoid	3*4	+
<i>Debaromyces</i> spp. M41	spherical	6.6	–
<i>Cryptococcus</i> spp. M44	Spherical slightly elongated	7*5.5	+
<i>Kloeckera</i> spp. M55	Ovoid	10*6.5	–
<i>Rhodotorula</i> spp. M59	oval elongated (cylindrical)	15.6* 3.8	+ / –
<i>Candida</i> spp. M65	oval elongated	5*2.5	–

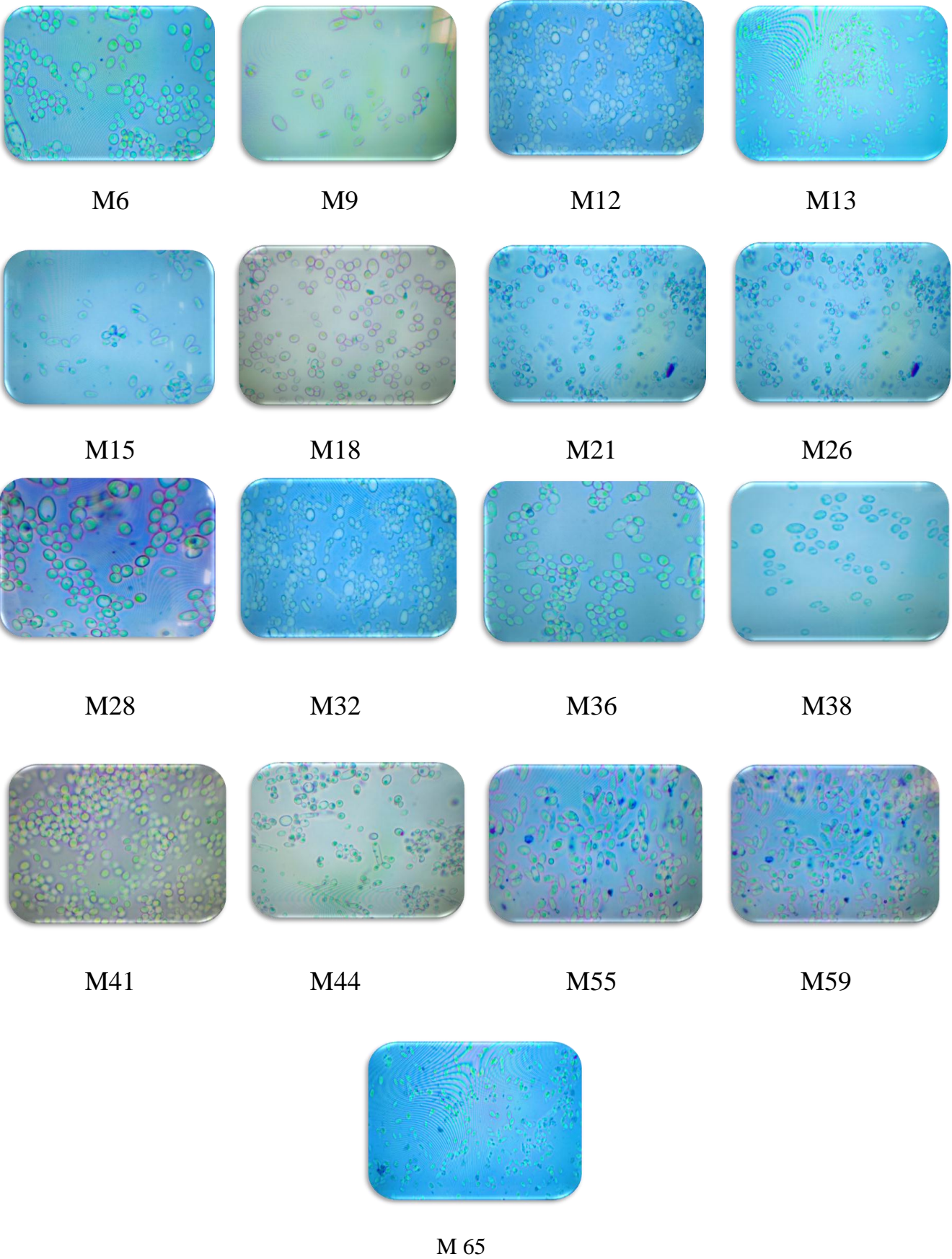


Fig. 2: Shapes of Isolated Yeasts Cells at 40X Magnification

Diazonium Blue B (DBB) Color Test

The results showed that among the 17 tested yeast isolates, only one isolate of *R. mucilaginosa* M59 (5.88%) was positive for this test, while the remaining 16 isolates were negative (94.11%) Fig. (3). These characteristics are consistent with Kurtzman and Fill (1998). He mentioned that this test is widely used in distinguishing between Ascomycetes and Bazidomycetes.



Fig. (3): Diazonium Blue B (DBB) Color Assay of Isolated Yeasts Colonies Grown on YM Agar for 10 Days

Growth in 25°C and 37°C Test

The results of this test showed the ability of all 17 (100%) isolates of yeast under study to grow at a temperature of 25°C, while the results showed the ability of 12 (70.58 percent) isolates to grow at 37°C, as 4 isolates grew weakly (*C. tropicalis* M9, *Klo. apiculata* M55, *Z. rouxii* M32 and *C. magnoliae* M36) with a percentage of 23.52%, while 5 isolates did not show a clear response to growth at this temperature (a percentage of 29.41%). (*P. anomala* M6, *P. membranaefaciens* M28, *Crypto. laurentii* M44, *Klo. apiculata* M55 and *C. rugosa* M65) (Table 4). The present results are similar to what Kirsop and Kurtzman (1988) and Malla Obaeda (2017) mentioned during their study of a group of yeasts isolates at various temperatures.

Assessing Ability to Utilize Nitrate as a Sole Nitrogen Source

It was noted that 11 (64.70%) isolates were able to benefit from nitrate as the only source of nitrogen, despite the variation in the ability to benefit from it. The remaining isolates (6 and 35.29%) were negative for the test (Table 4). This is consistent with the findings of Malla Obaeda (2020) research of Mesopotamia sciences and Ebabhi *et al.* (2013) research of cassava and maize when diagnosing a group of yeasts.

Assessing Preservative Resistance of Glacial Acetic Acid

The results of this test came to show that 8 (47.05%) isolates were positive for this test, while the remaining 9 isolates (52.94%) showed a negative result for this test, (Table 4). This is consistent with what was mentioned by Pitt and Hocking (2009).

Growth at Reduced Water Activities in High Carbohydrate Levels

The present results revealed that 12 (70.58%) isolates were negative for the test, while 5 (29.41%) isolates were positive for the test.

Growth at Reduced Water Activities and High Level of Sodium Chloride

The results showed that 7 (41.17%) isolates were negative for the test, while 10 (58.82%) isolates were positive for the test. This result approximates what was reported by Pitt and Hocking (2009).

Table 4: Biochemical diagnostic tests for the yeasts isolates under study

Diagnosed Yeasts	Test Type						DBB Test*
	Ability to grow in						
	25°C	37°C	Presence of nitrate as a source (N)	Presence of glacial acetic acid	Low water level high carbohydrate	Low and high-water level of NaCl	
<i>P. anomala</i> M6	+++	–	+++	–	+	–	□
<i>C. tropicalis</i> M9	+++	+	–	–	+	+	□
<i>Kluy. lactis</i> M12	+++	+++	–	–	+++	–	□
<i>C. albicans</i> M13	+++	+++	+	+++	++	+++	□
<i>C. utilis</i> M15	+++	+++	+++	+++	+	+	□
<i>G. candidum</i> M18	+++	+	+++	–	++	+	□
<i>C. krusei</i> M21	+++	+++	+++	+++	+++	+++	□
<i>S. cerevisiae</i> M26	+++	+++	–	++	–	–	□
<i>P. membranaefaciens</i> M28	+++	–	+	+++	–	–	□
<i>Z. rouxii</i> M32	+++	+	–	–	+++	+++	□
<i>C. magnoliae</i> M36	+++	+	+	–	+	+	□
<i>T. asahii</i> M38	++	+++	–	–	–	+	□
<i>D. hansenii</i> M41	+++	+++	+++	+++	+++	+	□
<i>Crypto. laurentii</i> M44	+++	–	+	+	–	–	□
<i>Klo. apiculata</i> M55	+++	–	+	–	–	–	□
<i>R. mucilaginosa</i> M59	+++	+++	++	+++	++	+++	■
<i>C. rugosa</i> M65	+	–	–	–	+	–	□

(–): no growth, (+): weak growth, (++) : medium growth, (+++) : good, dense growth : (■) Basidian yeasts, (□): cystic yeasts

*DBB= Diazonium Blue B.

CONCLUSION

In this study was isolated 71 local isolates and diagnosis yeasts from different plant sources, as the fruits were obtained from local markets in Mosul, Kirkuk, Erbil and Dohuk and from home gardens.

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عزل وتشخيص بعض انواع الخمائر من بعض النباتات

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مها بشار ابراهيم الطائي

قسم علوم الحياة / كلية العلوم / جامعة الموصل

الملخص

تضمنت الدراسة الحالية عزل 71 عذلة محلية من الخمائر خلال ثلاثة أشهر ابتداء من تاريخ 1/10/2021 ولغاية 1/1/2022 من مصادر نباتية مختلفة، إذ تم الحصول على الثمار من الاسواق المحلية في مدينة الموصل، كركوك، اربيل ودهوك ومن الحدائق المنزلية. وقد تم تشخيص العزلات بعد زراعتها على الوسط الغذائي الصلب (MEA) Malt Extract Agar Medium) بالاعتماد على الاختبارات المظهرية المتضمنة لون المستعمرة، شكلها، قطرها، طبيعة حافاتهما، ارتفاعها، قوامها، لمعانها وشكل سطحها والمزرعية والمجهريّة عند القوى 40X لملاحظة شكل الخلايا الخميرية وقياس أحجامها باستخدام العدسة العينية (Ocular (E 10X والكيموحيوية المتضمنة اختبار أزرق الديازونيوم، النمو في درجة حرارة 25°م و 37°م، قابلية الاستفادة من النترات كمصدر وحيد للنيتروجين، القدرة على مقاومة حامض الخليك الثلجي، قابلية النمو في المستويات المنخفضة من الماء مع الارتفاع في مستوى الكربوهيدرات، قابلية النمو في المستويات المنخفضة من الماء مع زيادة مستوى كلوريد الصوديوم وقدرة الخمائر على تشكيل المايسليوم. وظهرت النتائج انها تعود الى 17 نوع مختلفا من الخمائر *Debaromyces*, *Saccharomyces*, *Kloeckera*, *Geotrichum*, *Kluyveromyces*, *Candida*, *Pichia*, *Rhodotorula*, *Trichosporon* و *Zygosaccharomyces*, *Cryptococcus*.

الكلمات الدالة: الخمائر، العزل، التشخيص، المظهري، الاختبارات الكيموحيوية.