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Effect of Some Ecological Factors on Occurrence of Yeasts in Soil and Sediment from Iraq

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ABSTRACT

Soil is one of the favorable habitat for microorganism. It considered being the home of wide range of them in particularly, bacteria and fungi. Southern Iraq is characterized by a multiplicity of ecosystem, which include desert, agricultural and marine areas, each of which has special composition that differs from the neighboring system, whether in terms of vegetation or various environmental factors. This in diversity, in turn, may lead to microbial diversity that can be used in different biotechnologies. Regarding that little is known about yeast diversity in such habitats, and therefore the current study aims to assess the yeast community in soil and sediments samples from Basrah and Dhi-Qar provinces, Southern Iraq. Thirty-one species belong to 19 genera were encountered. The isolated species consist of 16 species of Ascomycota and 15 species of Basidiomycota. The soil of Basrah and Dhi-Qar support the growth of diverse species belonged to the genera *Aureobasidium*, *Cutaneotrichosporon*, *Debaryomyces*, *Filobasidium Geotrichum*, *Hanseniaspora*, *Lodderomyces*, *Meyerozyma*, *Symmetrospora*, *Torulaspora*, *Vishniacozyma*, *Pichia*, *Yarrowia*, *Cystobasidium*, *Galactomyces*, *Rhodotorula*, *Wickerhamomyces*, *Candida* and *Naganishia.* One hundred and twelve fungal isolates were identified using the conventional methods depending on morphological characteristics. CHROMagar candida was used as differential culture medium. Iodine stain was used to differentiate ascospores and basidiospores. In addition biochemical method represented by VITEK was used as well as molecular identification. This study represents the first report of occurrence of yeast species in soil and surface sediment samples from Basrah and Dhi-Qar provinces, Southern, Iraq, with effect of some ecological factors on isolation yeast from different location.

1. Introduction

Soil is one of the favourable habitat for microorganisms. It considered to be the home of wide range of them in particularly, bacteria and fungi soil inhabitant organism participant in many soil functions such as absorbing neutralizing and transforming compounds that may harmful to the environment (Fouzia & Amir, 2011). In the 19th century, pioneer studies reported the present of yeast in sources found underground. The soil then was considered as reservoir of yeasts that present in environment above it. Later on, studies found that yeasts live in soil are taxonomically different from the yeasts live above soil, moreover, soil yeasts have been adapted to a wide range of environmental conditions (Yurkov, 2018). Yeast are an extra taxonomic group consist mainly from unicellular micromycetes that lost the mycelial structure (Glushakova et al., 2019).

They are single-celled beings, like molds and mushrooms they are classified as members of the Kingdom of Fungi. Furthermore, despite their unicellular structure they have a well-developed cellular organization comparable to the organization found in beings such as human, particularly. The genetic content inside the nucleus (Thapa et al., 2015).

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They are saprotrophs that assimilate organic compounds derived from plants or animals, Additionally, they are decomposer on the other hand , some species of yeasts are pathogenic either to plants or animals-Yeast facilitate number of biological processer such as food fermentation and alcoholic beverages, secondary metabolites production including vitamins, organic acids, carotenoid and recombinant vaccine (Jamali et al., 2016).

Yeast in habitat a wide range of various soil types from cultivable lands to forest, in addition to soils found in environments with extreme conditions such as Antarctica. The diversity of yeasts in soils can be affected by several factors including temperature, humidity, geographical locations and chemical composition. Despite the existence, knowledge regarding yeast living in soils it still considered as limited (Moreira & do Vale, 2018). Despite a number of studies that deal with mycobiota inhabiting soil and sediments in wetlands, desert and arid regions of Southern Iraq, none of them were exposed to the yeast community in those habitats and the majority of previous studies concerned with filamentous fungi. Therefore, this study aimed to examine the occurrence of yeast species inhabiting the soil and aquatic sediments in two provinces, Southern Iraq.

2. Materials and Method

2.1. Samples Collection and Description of Study Areas

Seventy-five soil or aquatic sediments samples were collected during the period October 2017-Augest 2018 from 17 sites in Basrah and Dhi-Qar governorate, Southern Iraq (Figure 1). Surface sediments samples were collected with an Ekman grab. Samples were stored in polyethylene bags and kept under refrigeration until processed within one to two days. Description of sampling sites and geographical coordinates are shown in Figure 1 and Table 1.

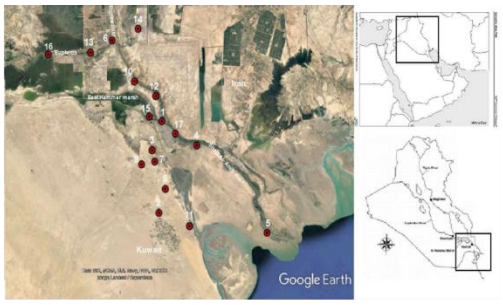

Figure 1: A map of Basrah and Thi -Qar provinces southern Iraq illustrating the 17 sampling sites 2017/ 2018.

1. Karmat Ali	2.Sufwan	3.Al-Shaiba	4.Abu-Alkhaseeb	5.Al-Faw
6.Al-Qarna	7.Al-Zubair	8.Khor-Al-zubair	9.Al-Bargesia	10.Al-Dair
11.Om-Qaser	12. Al-Hartha	13.Al-Midaina	14.Majnoon oil field	15.East Hammar Marsh – Al-Sadah
16.Al-Chibayish	17.Shatt Al-Arab			

Table 1
Description of sampling sites and geographical coordinates location of the study area

Areas of study	Location according to value of Global position system	Sample numbers
Karmat Ali area: It is located on the South of the Karma Ali River, opposite the Naval Academy, and This area is located north of Basra	30.5862° N, 47.7576° E	1.
Sufwan is a town in Southeast Iraq on the border with KuwaitIt is 49 km from the center of Basra Governorate It is located to the west of Basra Governorate	30.1097° N, 47.7194° E	2.
Al-Shaiba The city of Al-Shuaiba is located 11 kilometers northwest of Al-Zubair District and to the west of the Basra Governorate Center	30°26′14″ N 47°41′5″E	3.
Abu-Alkhaseeb an Iraqi city located South of Basra Governorate and extends over The right bank of the Shatt al-Arab, with a distance of 16 km	30.27′ 11°N 47.59 ′47°E	4.
Al-Faw is located in the South of Basra Governorate, about 100 km away	29.9812° N, 48.4676° E	5.
Al-Qarna The city is located at the confluence of the Tigris and Euphrates rivers, and it is 74 km2 from Basra.	31.0174° N, 47.4245° E	6.
Al-Zubair is located Southwest of the city of Basra in Southern Iraq	30.3770° N, 47.7008° E	7.
Khor-Al-zubair Is an esturine / lagoonal environment situated at the head of Arab Gulf , 30 km South Southest of Basrah	30.2331° N, 47.7687° E	8.
Al-Barjisiya is an Iraqi sub-district located in Basra Governorate, Southern Iraq, administratively affiliated to Al-Zubair district	30.3655089,N 47.6086213.E	9.
Al-Dair It is an Iraqi district located in the northern part of Basra Governorate, along the coast of the Shatt al-Arab	47.5742088E 30.7987835N	10.
Umm Qasr is an Iraqi city located in the far South of Iraq wiDhin the Basra Governorate	30°2′03″N 47°55′46″E	11.
Al-Hartha is an Iraqi city and a sub-district located in the north-east of Basra Governorate on the eastern bank of the Euphrates River, bordered on the north by Qurna district and to the South by the center of Basra Governorate	30.7186 N 47.7208 E	12.
Al-Midaina This district is located in the northwest of the Basra governorate on the western bank of the Euphrates River, bounded to the east by the Qurna district and to the west by the Dhi Qar governorate in the north by the Maysan governorate in the South by the Zubair district	30° 57' 28.77"N 47° 16' 18.94 E	13.
Majnoon oil field are oil fields located in the east of Basra Governorate near the Iran-Iraq border, and it is 60 km away from the city of Basra	31.0738° N, 47.6106° E	14.
East Hammar Marsh – Al-Sadah This marsh is located South of the Euphrates River in Iraq, and extends from Souq al-Shuyukh in the west to the outskirts of Basra at the Shatt al-Arab in the west.	30° 50' 23.99" N 46° 53' 11.99" E	15.
Al-Chibayish District is one of the districts of Dhi Qar Governorate, located east of the city of Nasiriyah, northwest of Basra Governorate.	30.9555° N, 46.9757° E	16.
Shatt Al-Arab a river that forms at the confluence of the Tigris and Euphrates rivers, where the Tigris River meets the upper course of the Euphrates in the city of Qurna.	30° 31' 11" N 47° 50' 59" E	17.

2.2. pH and Salinity Measurement

Aqueous extract for each soil and sediment sample was prepared to measure the electrical conductivity and pH for each sample and this was done depending on (Estefan, 2013).

2.3. Isolation of Yeasts

From each sample, a series of dilution was made (10^{-1} - 10^{-3}), and one ml of each suspension (triplicates) were cultured on different growth media. Six types of culture media were used for isolation of yeasts, namely CHROM agar Candida (Himedia, India), Malt extract agar (MEA) (Himedia, India), Orange serum agar (Graumlich, 1981) Dichloran rose bengal agar (Fluka analytica,India), YL medium (Yeast extract malt extract agar+ crystal violet 0.01%) (Kurtzman et al., 2003) (Yeast extract peptone dextrose agar) (YEPDA) Malt extract agar 2 % and Potato tip medium for ascospores and basidiospores formation (Kurtzman et al., 2003). Each medium was supplemented with 50 µg /L chloramphenicol to inhibit bacterial growth. The Petri plates for all media were incubated at 25 C ◦ for 72 h. To obtain pure cultures, single colonies were isolated on a PDA medium. The pure isolates were sub cultured in slant of PDA for species identification.

2.4. Identification of Yeasts

The isolated pure yeasts were identified to genus and species level by classical methods, which included the following tests, germ tubes, arthroconidia, hyphae, chlamydospores, ascospores and basidiospores production, Diazonium blue B reaction growth on CHROM agar candida (Bio-Merieux,Lyon,France); biochemical reactions by Vitek2 system (Kurtzman et al., 2003). The identification was confirmed by using molecular techniques (Abu-Mejdad & Al-Badran, 2019).

3. Results and Discussion

3.1. Ecological Factors for Study Sites in Basrah and Dhi Qar

During the current study, a total of 112 pure isolates of yeasts were obtained from the different soil samples, the pH and salinity of the isolation site were measured where the range of salinity between (0.2-42.9 ppt) while pH was between (3.5-8.30) as shown in Table 2.

Table 2
pH and salinity of soil extract for study sites

Sample No.	Study site	PH	Salinity /ppt
1	Karmat Ali	6.08	21.5
2	Sufwan	6.87	0.6
3	Al-Shiaba	6.29	41.3
4	Abu-Al-Khasseeb	8.30	3.2
5	Al-Faw	6.30	42.9
6	Al-Qurna	3.5	6.5
7	Al-Zubair	7.39	1.4
8	Khor-AlZubair	7.87	2.5
9	Al-Bargesia	6.67	2.5
10	Al-Dair	6.61	10.3
11	Om-Qaser	6.89	1.7
12	Al-Hartha	8	1.8
13	Al-Midaina	6	1.5
14	Majnon oil field	6.2	0.2
15	East Hammar Marsh	5.9	10.1
16	Al-Chibayish	7.49	2,9
17	Shatt Al-Arab	7.5	5.01

During the current study, 112 yeasts isolates were recorded. All of these isolates were identified based on phenotypic and biochemical characteristics, and the diagnosis was confirmed by molecular analysis. These strains represent 31 different species belonging to 19 genera (Table 3 and 4).

Table 3
Species of ascomycetous and basidiomycetous yeasts with percentage of occurrence

No.	Genera	Species	No. of Isolates	%Occurrence
1	*Aureobasidium**	*A. melanogenum*	1	0.89
		C. tropicalis	11	10
22	*Candida**	*C. membranifaciens*	5	4.46
		C. glabrata	1	0.89
33	*Cutaneotrichosporon***	*C. dermatis*	1	0.89
44	*Cystobasidium***	*C. benDhicum*	3	2.67
		C. minutum	1	0.89
55	*Debaryomyces**	*D. hansenii*	8	7.14
66	*Filobasidium***	*F. oeirense*	1	0.89
77	*Galactomyces**	*G. pseudocandidum*	1	0.89
		G. reessii	1	0.89
88	*Geotrichum**	*G. candidum*	2	1.78
99	*Hanseniaspora**	*H. uvarum*	2	1.78
110	*Lodderomyces**	*L. elongisporus*	2	1.78
111	*Meyerozyma**	*M. caribbica*	3	3
		N. adeliensis	1	0.89
		N. albida	4	4
		N. albidosimilis	2	1.78
112	*Naganishia***	*N. diffluens*	7	6.2
		N. liquefaciens	2	1.78
		N. uzbekistanensis	1	0.89
		N. vishniacil	1	0.89
13	*Pichia**	*P. fermentans*	3	3
114	*Rhodotorula***	*R. diobovata*	3	3
		R. mucilaginosa	15	13.39
115	*Symmetrospora***	*S. folicola*	1	0.89
116	*Torulaspora**	*T. delbrueckii*	2	1.78
117	*Vishniacozyma***	*V. carnescens*	1	0.89
118	*Wickerhamomyces**	*W. anomalus*	1	0.89
		W. onychis	1	0.89
119	*Yarrowia **	*Y. lipolytica*	22	20

*Ascomycetous genera
** Basidiomycetous genera

Among the isolated taxa, twelve genera were assigned to ascomycota with 16 species, while seven genera belonged to basidiomycota with 15 species. *Yarrowia lipolytica* was the most frequent species with 20 % followed by *Rhodotorula mucilaginosa* and candida *tropicalis* (10%). Most of the remaining species revealed low occurrence Table 2. *Naganishia* was represented by seven species and *N.diffluens* was the most frequent species among the genus. *Candida* was second in the number of species recorded and was represented by three species, while the rest of fungal genera were represented by two or one species. Table 2. 18 species are a new record for Iraq mycobiota.

A total of 112 isolates were first identified depending on the morphological characteristics of the growing colonies on the PDA and YPEGA media, where appeared the different isolates variation in color and texture after the yeast isolates were purified, the growth test on CHROM agar candida depended on color identification did not give conclusive results . While the MEA 2% appeared high efficiency in producing ascospores or basidiospores as shown just 112 isolates.

Table 4
Morphological characteristics of yeasts

No	Species	No of isolates	Color	Shape	Elevation	Texture	Consistency	Colour on chrom agar Candida
1	*Aureobasidium melanogenum*	100	*Black*	*Hyphae and chlamydospore and yeast cells*	*No*	*Smooth*	*Slimy*	Green
2	*Candida tropicalis*	10	*White*	*Round to oval*	*No*	*Smooth*	*Butyrous*	blue
3	*C. membranifaciens*	2	*White*	*Ovoid to cylindrical*	*Elevate*	*Smooth*	*Butyrous*	Green
4	*C. glabrata*	25	*Cream*	*spherical*	*Elevate*	*Smooth*	*Butyrous*	White
5	*Cutaneotrichosporon dermatis*	20	*Cream*	*Yeast cell with athroconidia*	*Elevate*	*Fine wrinkled*	*Butyrous*	Dark- brown
6	*Cystobasidium benDhicum*	20	*Pinkish*	*Ovoid cells*	*No*	*Smooth*	*mucilaginous*	Green
7	*C. minutum*	*30*	*light pink*	*subglobose*	*No*	*smooth*	*Mucoid*	*Light Rose*
8	*Debaryomyces hansenii*	*3*	*yellowish-white*	*spherical*	*Elevate*	*Smooth*	*Butyrous*	*purpule*
9	*Filobasidium oeirense*	*90*	*pinkish-cream*	*sub globose or broadly ellipsoidal*	*No*	*Smooth*	*Mucoid*	*Dark-rose*
10	*Galactomyces pseudocandidum*	*5*	*White*	*rectangular arthroconidia Chlamydospores*	*Elevate*	*finely hairy*	*dry, and powdery*	*Aqueouse- white*
11	*G. reessii*	*14*	*White*	*rectangular arthroconidia Chlamydospores*	*No*	*Hairy*	*dry, and powdery*	*violet*
12	*Geotrichum candidum*	*5*	*White*	*arthroconidia*	*No*	*Soft*	*dry, and powdery*	*Rose*
13	*Hanseniaspora uvarum*	*40*	*Slightly brown*	*spheroidal to ovoid*	*Elevat*	*Smooth*	*Glossy*	*violet*
14	*Lodderomyces elongisporus*	*100*	*Tannish-white*	*the yeast cells spherical but they are usually ellipsoidal to elongate*	*Elevate*	*Smooth*	*Butyrous*	*Green*
15	*Meyerozyma caribbica*	*3*	*Tannish white*	*Ovoid*	*Elevate*	*Smooth*	*Butyrous*	*Violet*
16	*Naganishia adeliensis*	*200*	*white to cream*	*spherical, subglobose or ovoid*	*Elevate*	*Smooth*	*Mucoid*	*Rose*
17	*N. albida*	*1*	*Creamy*	*subglobose*	*Elevate*	*Smooth*	*Butyrous*	*Dark -brown*
18	*N. albidosimilis*	*20*	*cream to rose*	*Ovoid*	*No*	*Smooth*	*Mucoid*	*violet*
19	*N. diffluens*	*300*	*Cream*	*Sub globos*	*Elevate*	*Smooth*	*Mucoid*	*violet*
20	*N. liquefaciens*	*10*	*cream to pinkish-cream*	*Sub globose*	*Elevate*	*Smooth*	*Mucoid*	*violet*
21	*N. uzbekistanensis*	*3*	*white to pinkish*	*Sub globose*	*Elevate*	*Smooth*	*Mucoid*	*Greenish violet*
22	*N. vishniacil*	*1*	*Cream*	*broadly ellipsoidal*	*Elevate*	*Smooth*	*Butyrous*	*Bluish-violet*
23	*Pichia fermentans*	*2*	*Tannish*	*Ovoid*	*Elevate*	*Wrinkled*	*Butyrous*	*Light violet*
24	*Rhodotorula diobovata*	*100*	*orange-red to dark-red.*	*Ovoid*	*Elevate*	*Smooth*	*Mucoid*	*Light violet*
25	*R. mucilaginosa*	*2*	*saffron-orange to deep cora*	*Sub globose*	*Elevate*	*Smooth*	*Mucoid*	*Light rose*
26	*Symmetrospora folicola*	*50*	*Red*	*ellipsoidal*	*Elevate*	*Smooth*	*Butyrous*	*rose*
27	*Torulaspora delbrueckii*	*100*	*Tannish white*	*spherical*	*Elevate*	*Smooth*	*Butyrous*	*Rose*
28	*Vishniacozyma carnescens*	*1*	*pale yellowish brown to grayish cream,*	*ellipsoidal*	*Elevate*	*Smooth*	*butyrous to mucoid*	*Green*
29	*Wickerhamomyces anomalus*	*2*	*white to tannish-white*	*spherical*	*Elevate*	*Smooth*	*Butyrous*	*Light violet*
30	*W. onychis*	*20*	*Cream*	*Ovoid*	*Elevate*	*Smooth*	*Butyrous*	*White*
31	*Yarrowia lipolytica*	*30*	*White*	*spherical*	*Elevate*	*Smooth*	*Butyrous*	*Green*

All tested isolates (N= 112) gave positive results with brown color for ascospores (hat shape) of *Wickerhamomyces anomalus* and basidia with basidiospores of Filobasidium *oeirense*as showed *staining with* iodine stain.

Table 5
Phenotypic, biochemical and molecular identification of yeast

Isolate No.	Chromagar candida	Vitek2 identification	percentage	Molecular identification	Blast homology
1.	*Candida albicans*	Un identified	-	*Aureobasidium melanogenum*	100%
2.	*C. tropicalis*	*C. tropicalis*	96%	*Candida tropicalis*	100%
3.	*C. albicans*	Un identified	-	*C. membranifaciens*	100%
4.	*C.parapsilopsis*	*C. tropicalis*	96%	*C. glabrata*	100%
5.	Other	*Trichosporon asahii*	88%	*Cutaneotrichosporon dermatis*	100%
6.	*C albicans*	Un identified	-	*Cystobasidium benDhicum*	99%
7.	*C.kruzi*	Un identified	-	*C. minutum*	98%
8.	*C.kruzi*	*C. famata*	99%	*Debaryomyces hansenii*	100%
9.	*C.kruzi*	*C. laurentii*	91%	*Filobasidium oeirense*	100%
10.	*C.parapsilopsis*	*C. famata*	95%	*Galactomyces pseudocandidum*	100%
11.	Other	*C. famata*	95%	*G. reessii*	100%
12.	*C.kruzi*	*C. famata*	95%	*Geotrichum candidum*	100%
13.	Other	Un identified	-	*Hanseniaspora uvarum*	100%
14.	*Candida albicans*	*C. famata*	95%	*Lodderomyces elongisporus*	100%
15.	Other	Un identified	-	*Meyerozyma caribbica*	100%
16.	*C.kruzi*	*C. famata*	95%	*Naganishia adeliensis*	99%
17.	Other	*C. laurentii*	96%	*N. albida*	100%
18.	Other	*C. laurentii*	96%	*N. albidosimilis*	99%
19.	Other	*C. albidus*	96%	*N. diffluens*	100%
20.	Other	*C. laurentii*	96%	*N. liquefaciens*	100%
21.	*Candida albicans*	*C. laurentii*	96%	*N. uzbekistanensis*	100%
22.	*C. tropicalis*	*C. tropicalis*	96%	*N. vishniacil*	100%
23.	Other	*Candida krusei*	93%	*Pichia fermentans*	99%
24.	Other	*C. laurentii*	96%	*Rhodotorula diobovata*	100%
25.	*C.kruzi*	*C. albidus*	93%	*R. mucilaginosa*	100%
26.	*C.kruzi*	Un identified	-	*Symmetrospora folicola*	100%
27.	*C.kruzi*	Un identified	-	*Torulaspora delbrueckii*	100%
28.	*Candida albicans*	Un identified	-	*Vishniacozyma carnescens*	100%
29.	Other	Un identified	-	*Wickerhamomyces anomalus*	100%
30.	*C.parapsilopsis*	Un identified	-	*W. onychis*	100%
31.	*Candida albicans*	*Malassezia furfur*	97%	*Yarrowia lipolytica*	100%

Table 6
Growth of 31 strains on six different culture media

No.	Species	DRBA(1)	MEA(2)	MEA2% (3)	OSA(4)	YL(5)	YEPDA(6)	15 C	25 C	37 C
	Aureobasidium. Melanogenum	+	+	+	_	_	+	-	+	+
	Candida. tropicalis	+	+	+	_	_	+	-	+	+
	C. membranifaciens	+	+	+	_	_	+	-	+	+
	C. glabrata	+	+	+	_	_	+	-	+	+
	Cutaneotrichosporon. dermatis	+	+	_	_	_	+	-	+	+
	Cystobasidium. benDhicum	+	+	_	_	_	+	+	+	-
	C. minutum	+	+	_	_	_	+	-	+	+
	Debaryomyces. hansenii	+	+	+	_	_	+	-	+	+
	Filobasidium. oeirense	+	+	_	_	_	+	-	+	+
	Galactomyces. pseudocandidum	+	+	+	_	_	+	-	+	+
	G. reessii	+	+	+	_	_	+	-	+	+
	Geotrichum. candidum	+	+	+	_	_	+	-	+	+
	Hanseniaspora. uvarum	+	+	+	_	_	+	-	+	+
	Lodderomyces. elongisporus	+	+	+	_	_	+	-	+	-
	Meyerozyma. caribbica	+	+	+	_	_	+	-	+	+
	Naganishia. adeliensis	+	+	_	+	_	+	+	+	+
	N. albida	+	+	_	+	_	+	-	+	+
	N. albidosimilis	+	+	_	+	_	+	-	+	+
	N. diffluens	+	+	_	+	_	+	+	+	-
	N. liquefaciens	+	+	_	+	_	+	+	+	+
	N. uzbekistanensis	+	+	_	+	_	+	+	+	+
	N. vishniacil	+	+	_	+	_	+	+	+	+
	P. fermentans	+	+	+		_	+	-	+	+
	Rhodotorula. diobovata	+	+	_	+	_	+	-	+	+
	R. mucilaginosa	+	+	_	+	_	+	-	+	-
	Symmetrospora. folicola	+	+	_	_	_	+	-	+	+
	Torulaspora. delbrueckii	+	+	+	_	_	+	-	+	+
	Vishniacozyma. carnescens	+	+	-	_	_	+	-	+	+
	Wickerhamomyces. anomalus	+	+	+	_	_	+	-	+	+
	W. onychis	+	+	+	_		+	-	+	+
	Yarrowia. lipolytica	+	+	+	_	+	+	-	+	+

DRBA: Dichloran rose Bengal agar
MEA: Malt extract agar
MEA: Malt extract agar 2%
OSA: Orange serum sagar
YL: Yeast extract malt extract agar with 0.01 % crystal violet
YEPDA: Yeast extract pepton dextrose agar
+: growth
-: no growth

3.2. Growth Temperature

Shows that all isolated species were grown at 25 °C, while at 37°C all species were grown except for six, namely *Cystobasidium benDhicum, Loderomyces elongisporus, Naganishia diffluens, Rhodotorula mucilaginosa and Yarrowia lipolytica*. The lowest appearance of growth was at 15°C, and it represented only six species, vis *Cystobasidium benDhicum, Naganishia adeliensis, Naganishia albidosimilis, Naganishia liquefaciens, Naganishia uzbekistanensis* and *Naganishia vishniacil* (See Table 6).

Table 7
Comparison between yeasts in sediment and soil samples

species	Collection sites	Sample type	Sample numbers
L. elongisporus	Karmat Ali	Arable soil	S1
Y. lipolytica	Karmat Ali	sandy soil	S2
Y. lipolytica	Sufwan	Sandy soil	S3
R. diobovata	Al-Faw-Al-Nagaah	Surface sediment	S4
C. tropicalis	Al-Faw- Qishlah	Surface sediment	S5
Y. lipolytica	Al-Faw-Saffy	Surface sediment	S6
Y. lipolytica	Al-Hammar Marshes-Al-Sadah	Surface sediment	S7
C. minutum	Al-Hammar Marshes- Al-Sadah	Surface sediment	S8
G. pseudocandidum	Al-Hammar Marshes- Al-Burgha	Surface sediment	S9
Y. lipolytica	Al-Bargesia	Arable soil	S10
C. benDhicum	Chebiash marshes / Abo-Subat	Surface sediment	S11
Y. lipolytica	Al-Shaiba	Sandy soil	S12
R. mucilaginosa	Karmat Ali	Arable soil	S13
	Al-Shaiba	sandy soil	S14
C. membranifaciens *M. caribbica*	Al-Hammar Marshes /Al-Nagarah	Surface sediment	S15
R. diobovata	Al-Hammar Marshes /Al-Sadah	Surface sediment	S16
N. diffluens *N. liquefaciens*	Al-Hammar Marshes /Al-Salal	Surface sediment	S17
G. reessii	Al-Midaina	Arable soil	S18
C. tropicalis *G. candidum* *D. hansenii*	Al-Faw	Surface sediment	S19
F. oeirense *H. uvarum*	Abu-Alkhaseeb	Surface sediment	S20
Y. lipolytica	Al-Midaina	Arable soil	S21
Y. lipolytica	Al-Faw-Saffy	Surface sediment	S22
Y. lipolytica	Abu-Alkhaseeb	Surface sediment	S23
Y. lipolytica	Al-Hammar Marshes /Al-Fayhaa	Surface sediment	S24
Y. lipolytica	Al-Faw-Saffy	un cultivated soil	S25
C. membranifaciens	Abu-Alkhaseeb	Arable soil	S26
Aureobasidium. melanogenum *V. carnescens*	Al-Faw	Surface sediment	S27
N. albida	Karmat Ali	Arable soil	S28
N. diffluens *D. hansenii*	Al-Faw	Surface sediment	S29
N. adeliensis *N. albidosimilis*	Al-Midaina	Arable soil	S30
N. diffluens *L. elongisporus*	Al-Qarna	Arable soil	S31
N. liquefaciens *R. diobovata*	Karmat Ali/Shat Ai –Arab/Mhmudia	Surface sediment	S32
N. diffluens	Al-Faw -Race Al-Byshia	Surface sediment	S33
C. benDhicum *W. onychis*	Al-Zubair	Arable soil	S34
C. tropicalis *P. fermentans* *C. glabrata*	Al-Faw	Surface sediment	S35
N. albida *G. candidum*	Khor-Al-zubair	Surface sediment	S36
C. membranifaciens *C. dermatis*	Abu-Alkhaseeb	Arable soil	S37
N. albidosimilis	Al-Bargesia	Sandy soil	S38
D. hansenii	Al-Dair	Arable soil	S39
D. hansenii *N. diffluens* *N. vishniacil* *P. fermentans*	Al-Faw	un cultivated soil	S 40
C. tropicalis *D. hansenii* *N. diffluens* *N. uzbekistanensis* *P. fermentans* *S. folicola*	Al-Faw	Surface sediment	S41
C. tropicalis	Abu-Alkhaseeb	Arable soil	S42
Y. lipolytica	Al-Faw	Surface sediment	S43
H. uvarum *W. anomalus*	Chebiash marshes / Abo-Subat	Surface sediment	S44
C. tropicalis *D. hansenii*	Al-Hammar Marshes - Al-Sadah	Surface sediment	S45
R. mucilaginosa *T. delbrueckii*	Khor-Al-zubair	Surface sediment	S46
C. membranifaciens	Abu-Alkhaseeb	Arable soil	S 47
Y. lipolytica	Al-zubair	Arable soil	S 48
Y. lipolytica	Qurna-Hor-AlSaad	Arable soil	S 49
Y. lipolytica	Al-Midaina	Arable soil	S 50
D. hansenii *N. albida*	Safwan	Sandy soil	S 51
Y. lipolytica	Om-Qaser	Sandy soil	S 52
D. hansenii	Abu-Alkhaseeb	Arable soil	S 53
C. tropicalis	Al-Hartha	Arable soil	S54
R. mucilaginosa	Al-Faw	Surface sediment	S55
D. hansenii	Al-burga	Surface sediment	S 56
Y. lipolytica	Al-Midaina	Arable soil	S 57
Y. lipolytica	Al-Midaina	Arable soil	S 58
Y. lipolytica	Talha/ Al-Midaina	Arable soil	S 59

Y. lipolytica	Al-Ezz/ Al-Midaina	Arable soil	S 60
N. diffluens	Abu-Alkhaseeb	Arable soil	S 61
Y. lipolytica	Dear region	Arable soil	S 62
Y. lipolytica	Nashwa region	Arable soil	S 63
Y. lipolytica	Majnoon oil field	Sandy soil	S 64
Y. lipolytica	East Hammar Marsh – Al-Sadah	Surface sediment	S 65
R. mucilaginosa	Al-Sabbaghia Al- Chebiash	Surface sediment	S 66
Y. lipolytica	Shatt Al-Arab -Siba	Surface sediment	S 67
Y. lipolytica	Al-Hammar Marshes Al-Qishlah	un cultivated soil	S 68
Y. lipolytica	East Hammar Marsh Al-burgah	Surface sediment	S 69
Y. lipolytica	Abu-Alkhaseeb	Arable soil	S 70
Y. lipolytica	Shatt Al-Arab- Abu-Alkhaseeb	Surface sediment	S 71
Y. lipolytica	Al-baghdadya Chebiash	Surface sediment	S 72
Y. lipolytica	East Hammar Marsh Al-mafraq	Surface sediment	S 73
Y. lipolytica	Al-Qurna	Surface sediment	S 74
Y. lipolytica	Al-Qurna	Surface sediment	S 75

3.3. pH and Salinity

The results of this study showed that the pH of all samples were varied between 3.5-8.30, soil pH is the measure of acidity and alkalinity in soils. It normally falls between 3 and 10, with 7 being neutral. Ultra-acidic soils (pH < 3.5) and very strongly alkaline soils (pH > 9) are rare (Slessarev et al., 2016). The optimum pH for most microorganisms is near the neutral point (pH 7.0). Molds and yeasts are usually acid tolerant and are therefore associated with the spoilage of acidic foods. Yeasts can grow in pH value ranging between 4 and 4.5.

Soil pH scales could directly have an effect on fungal community composition by imposing a physiological constraint on fungal survival and growth. A few fungal taxa are unable to grow or survive if the soil pH falls outside the exact range. Fungi usually grow well in acidic conditions; however, some fungi grow well in neutral to slightly alkaline conditions. This is because, not all members of each phylum behave in the same way (Zhang et al., 2016).

For salinity, it was high in most sediment samples, possibly affected by the estuary of Shatt al-Arab that connected to the salty water Arabian Gulf. Whereas, marsh samples were less salinity may be due to the continuous washing of soil or because they were not connected to the dumping of sewage. Agricultural, sandy and clay soils were more less saline, which may be reasoned to dilution, that come from irrigation water or rain.

Our results showed that the emergence of many halophilic or halotolerance yeasts, which were mostly isolated from the surface sediment of different sites in Basra and Dhi-Qar provinces. The explanation of that, possibly due to the adaptation of these yeasts to such environmental conditions represented in salinity, low organic matter and vegetative cover as well as, using the seawater in preparing isolation media to provide environmental conditions similar to their original habitat. This finding is consistent with Chi et al., (2010). The different yeasts were isolated from different samples. The maximum of Yeast colonies were obtained from both Faw and Qurna soil samples, which is mostly occurrence in Basrah soil. It is also found that when yeast is grown in liquid medium, the culture follows a well established pattern for microbial growth. Cultures are usually started by inoculating media with a small number of cells.

3.4. Occurrence Percentage of Yeasts Isolates From Soil

During the present study, 112 yeast strains were identified molecularly. The results showed that the highest incidence (20%) of the yeast was *Yarrowia lipolytica*, which was more prevalent in the tested sandy, agricultural and surface sediment soils. The explanation of this result may be attributed to that *Y. lipolytica* is related to ascomycete's fungi that characterized by the formation of ascospores and pseudohyphae due to heat stress. In addition, yeast cells were adapted to stressful environments to become resistant for inappropriate conditions, or perhaps its ability to withstand the various environmental conditions was enhanced. Similar explanation was given by (Hackenschmidt et al., 2019). This species may be possessing a high enzymatic system that exploit various sources of food (Zieniuk & Fabiszewska, 2019).

As stated by (Coelho et al., 2010), some fungi have the ability of dimorphic growth i.e., in the mycelium form or yeast like, contingent upon the ecological conditions. A wide range of fungi including *Yarrowia lipolytica* possessing this dimorphism phenomenon, which is predominantly significant for modifying the cellular machinery extensively in reaction to environmental signals. Extremely variable conditions (fluctuations in the growth, gaseous atmosphere, temperature, pH and may be the existence of particular compounds in the culture media) may support inducing the transition of dimorphism phenomenon (mycelium-to-yeast or vice versa). Furthermore, *Yarrowia lipolytica* possess the ability of growing in the two forms (yeast-like and short mycelial cells) in the same time. Therefore, it can be clearly stated that *Y. lipolytica* under inappropriate environmental conditions may grow dimorphically to become resistant and prevalent in different environments.

Rhodotorula mucilaginosa was the second highest predominant species, which was found at 13.39% in the different soil samples. Similar results were obtained for the emergence of this carotenogenic yeast in Brazil and Eygept soils (El-Ziney et al., 2018). Species of the genus Rhodotorula can be isolated from different sources i.e., leaves tree, soils, meats, dairy products, pickle and traditional sweet. The occurrence of *R. mucilaginosa* in the second rank maybe attributed to their pigmented strains, which were more tolerance to UV compared to albino strains. In addition to, a high content of carotenoid in yeast cells during the stationary growth phase that enhanced yeast survival. It is confirmed that accumulation of torularhodin establishes an important mechanism for improving yeast resistance to UV-B. Moreover, it is probable that carotenoids can be associated with modification of membrane permeability and thus increased cellular resistance to oxidation and radiation (Kot et al., 2020).

Candida tropicalis and *Debaryomyces hansenii* were in the Dhird and fourth ranking with 10% and 7.14% respectively. These two species may be halophilic or halotolerance isolated from soils characterized with a high salt level, which provided favorable environmental conditions (Ekpenyong &

Asitok, 2019) compared to the 14 isolates that appeared with lowest rate (0.89%) for each. The reason of less percentage of such species may be due to high salinity in most studied samples. The emergence of these fungi for one time suggests non-spread of these fungi in the studied environment, especially those isolated from sediments that characterized with the low temperature and little concentration of oxygen. Whereas, the isolation of halophilic fungi in a number of samples with high frequencies can be attributed to their effectiveness and important roles in the studied environments for disintegration of plant and animal residues and reduction of organic matter, taking into account that some of these fungi have not been registered in the soil of Iraq.

Fungi in the cultivated saline soils appeared in small percentages because they were non-halophilic or non-saline-tolerant fungi. In addition , chemical treatments of agricultural crops such as spraying pesticides may be caused the lack of spread to some types of yeasts in the agricultural soils because their enzymatic activities affected by such chemicals (Baćmaga et al., 2015). All tested isolates (112) gave positive results with a brown color which clarify the appearance of ascospores and basidiospores. According to (Karakehian et al., 2019), the role of iodine is covalently linked to the glycogen components in yeast cell wall to become stained and also help in visibility of ascospores and basidiospores. After the staining of these reproductive structures with iodine, they become pale or refractile and the cytoplasm turns into yellow-gold color, while the glycogen material turn into brown. The iodine test used for glycogen detection is considered chemical reagent called Lugolos iodine, which give brown-blue color as reaction color.

The results of this study is almost in accordance with the findings above. Interestingly, during this study there was no soil sample free of yeasts. This indicates the wide spread of yeasts in the studied sites, perhaps due to suitable environmental conditions, especially temperature, abundance of nutrients and formation of some structures (chlamydospores, ascospores and basidiospores) which gave yeasts resistance to unsuitable conditions. After dilution plate method, the most common yeast obtained from these soils Yarrowia *lipolytica,* which was detected in most soil samples with various occurrence percentage compared to other yeasts i.e., *Rhodotorula mucilaginosa, Debaryomyces hansenii* and *Candida tropicalis*. Other researchers (Abu-Mejdad et al., 2019) also achieved these results

3.5. Morphological Identification

Morphological features are still of considerable importance in yeast taxonomy. During this study of phenotypic characteristics, this is the first identification of 112 yeast isolates started with the evaluation of macroscopic criteria, morphological characteristics of colonies on isolation media. These criteria make the authors confident to identify the big number of isolates presumably. The identified isolates were belonging to the genera *Cryptococcus, Rhodotorulla, Geotrichum, Aureobasidium, Candida …* ect. Other features observed on the colonies also were taken into account such as appearance and color. Microscopic features like pseudohyphae, blastoconidia, capsule, true hyphae and arthroconidia are necessary for primary identification.

CHROMagar Candida medium was used for yeasts identification depending on the observed different colors; however, this medium appeared to be poor for identification due to negative results such as indistinguishable colors; therefore, this medium was precluded from the identification of ecological isolates. This finding is corresponding with (Ghelardi et al., 2008) who mentioned the lack of discrimination observed for many *Candida* spp. that partially limits the usefulness of CHROMagar Candida in the clinical mycology laboratory. Therefore, this lack of discriminatory activity is a disadvantage of using for non-clinical samples. Furthermore, our results shown using MEA 2% and 5% for formation ascospores and basidiospores for all isolates appeared to be the best with high efficacy in formation reproductive structures.

3.6. Temperature

Temperature is one of the most important physical parameters which has a direct influence on yeast growth, however, the current analysis indicated that different yeast strains grew best at different temperatures (Salvadó et al., 2011). In our study, the effect of different temperatures on the vegetative growth of 112 isolates was tested. The optimum temperature for the growth of all tested isolate was 25 °C, most of the isolates grew at 37 °C and very few isolates grew weakly at 15 °C. It can be clearly seen that the most of isolated yeasts were fall within the terms of thermophilic, thermotolerance and mesophilic or mesotolerance. The reasons of these results may be attributed to the isolates were collected from regions known with their high temperatures. Therefore, the isolates that grew at 15 °C were little, which appeared after 30-60 days or perhaps the slow growth of these isolates due to the consumption of nutrient sources in the cultural media (Tiquia-Arashiro & Rodrigues, 2016). The media, DBRA, MEA, and YEPDA were positive in growth for all isolates of fungal species, while only MEA2 percent for Ascomycota fungi, either Orange serum agar for acidophilic *Rhodotorula* and *Naganishia* species, and Yeast extract malt extract agar with 0.01 % crystal violet is specific for only the type *Yarrwia lipolytica* (Abu-Mejdad, 2019).

4. Conclusion

The current study considered the first in isolating yeasts from Iraqi soil and revealed a biodiversity in soil samples studied from provinces Basrah and Dhi-Qar, beside of the pH and Salinity effect on variation species of yeast, which we can isolate it from sediment and soil in Basrah and Iraq.

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