ISRG Journal of Multidisciplinary Studies (ISRGJMS)

ISRG PUBLISHERS
Abbreviated Key Title: isrg j. multidiscip. Stud.
ISSN: 2584-0452 (Online)
Journal homepage: https://isrgpublishers.com/isrgjms/
Volume – II Issue – IX (September) 2024
Frequency: Monthly

Isolating yeasts from the water of different rivers in Basra and diagnosing them using the Vitek 2 compact system

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| **Received:** 12.09.2024 | **Accepted:** 16.09.2024 | **Published:** 22.09.2024

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Abstract

300 pure isolates of yeast isolated from 25 rivers dating back to different rivers in Basra Governorate / Iraq were isolated and diagnosed, and the sample collection period was from April 2014 - February 2015. These isolates were identified using modern technology, which is using the Vitek 2 Compact system technology, and the genera were What was obtained is as follows: Candida , Cryptococcus, Kloekera , Rhodotorula , Stephanoascus

The results showed that C. tropicalis is the most frequent among the isolated species, with a frequency rate of 36.3%, while C. lipolytica is the least frequent, recording a frequency of 0.7%.

As for the sites that showed the greatest presence of yeast, the Al-Maqal 1 site recorded an appearance rate of 84%, while the Medina site recorded the lowest appearance rate of 24%.

Keywords: *Yeasts, Candida, frequency ratio*

Introduction

Yeast is a eukaryotic microorganism from the kingdom of fungi, and it has about 1,500 species. Most of them reproduce asexually. People use yeast in baking and manufacturing alcoholic beverages.

Commercially used yeast consists of populations of microscopic, single-celled yeast organisms. Although there are more than 600 species of yeast, only a few have commercial uses.

Until 1876 AD, people used to make bread, beer, and wine without understanding or being aware of the role that yeast plays in making these products. In that year (1876 AD), the French scientist Louis Pasteur reported that yeast was a living organism and that it played an important role in the manufacture of beer.

DOI: 10.5281/zenodo.13824711

Yeast reproduces quickly and grows particularly well in a sugary environment. Yeast reproduces by mitosis (the division of one cell into two cells) or by budding. During budding, part of the cell wall swells and forms a new growth called a bud. This bud then separates and forms a new, independent cell. Yeast fungi lack chlorophyll, the green substance that plants use to make their food. Therefore, yeast depend on external sources for food. Yeast is fed with sugar produced from various natural sources such as fruits, grains, juices, and molasses. Yeast cells produce chemical compounds called enzymes, or fermenters, that have the ability to break down yeast food. Different types of yeast produce different types of enzymes. Some enzymes break down sugars into alcohol and carbon dioxide during the fermentation process (Balasubramanian et al. 2004).

The VITEK 2 COMPACT SYSTEM and compact device are ideal for biopharma companies that routinely perform microbial production. An automated system ensures excellence and improves your workflow. With its proprietary database, VITEK® 2 enables the diagnosis of a wide range of microorganisms. It is characterized by the following:-

- Optimized workflow: All stages are automated, from reading to recording results
- Full traceability: The risk of transcription errors is minimized

Advanced colorimetric technology™ for microbial identification

The efficiency of the VITEK® 2 compression system is based on advanced colorimetric technology: the system reads the latest generation VITEK test cards - containing 64 wells to ensure accuracy - every 15 minutes using three different wavelengths. With this technique, more data is analyzed, which increases the accuracy of the results.

Materials and methods

1. Collect and culture samples

During the study, 125 water samples and 125 mud sediment samples were collected from different rivers in Basra Governorate, including 25 rivers. The collection period was from April 2014 until February 2015, and the collection was five replicates for each river (water + mud sediments). The collection was for water samples. Using sterile glass bottles with a capacity of 100 ml, the sample was taken at a depth of 30 cm. As for the clay sediment samples, the collection was done using sterile nylon bags. The sample was taken from the river dredge from the surface of the soil once and from a depth of 30 cm again. The samples were planted according to the type of sample, where the aquatic samples were grown using the filtration method, by filtering 50 ml of the sample water using a filter paper (Cellulose nitrate filter) with a diameter of (0.45µm). After completing the filtration process, the filter papers were placed on plastic culture dishes containing the PDA culture medium. Then incubate the plates in the incubator at 30°C. As for the clay sediment samples, they were planted using the dilution method, where 1 gram of the sample was weighed and then placed in a tube containing 10 ml of sterile distilled water. After shaking the solution (dilution) well, 1 ml was taken from it and placed in the middle of a sterile culture dish, and then an amount of the medium was poured over it. Refrigerate the PDA at 45°C, and after the occlusions harden, place it in the incubator at 30°C.

2. Examination and Identification of specimen

The above-mentioned plates were examined after 48 hours of incubation to observe yeast growth, and the plates that did not contain yeast growth were discarded after five days (Brooks, et.al., 2007). The dishes were initially examined using an anatomical microscope, then the isolated yeasts were examined under an optical microscope by preparing clean glass slides, placing a drop of cotton blue lactophenol dye on them, transferring part of the growing yeast colony to the glass slide using a sterile needle, then placing the slide cover. The yeasts isolated during the current study were identified based on the following references:

(McGinnis, 1980; Ellis, 1994; Hoogde & Guarro, 1995; Sullivan, et al., 1998; Kurtzman & Fell, 1999). The yeast colonies were counted using a colony counting device, and then the isolates were transferred onto a tilted culture medium in sterile bottles, and they were also transferred into a liquid culture medium in sterile bottles, and finally they were transferred onto a semi-solid culture medium in sterile bottles, and all were stored in the refrigerator after being grown in the incubator until use. To complete the diagnosis.

3. Calculating the frequency and spatial occurrence of isolated yeasts:

Spatial Frequency and Occurrence of yeast isolates

The percentage frequency and occurrence of the yeast species isolated from the random study sites was calculated according to the following equations and according to what was mentioned by (Muhsin, 1985).

$$\text{Percentage of frequency} = \frac{Number\ of\ colonies\ of\ the\ genus\ or\ fungal\ species}{The\ total\ number\ of\ colonies\ of\ fungal\ genera\ or\ species} \times 100$$

$$\text{Impression percentage} = \frac{The\ number\ of\ samples\ in\ which\ the\ genus\ or\ fungal\ species\ appeared}{Total\ number\ of\ samples} \times 100$$

4. Diagnosis using the Vitek 2 compact system (Identification by)

The tubes for the device were prepared and placed in a special mold for the device called Cassete. These tubes were filled with 3 ml of the device's saline solution, and then a portion of the yeast colonies to be diagnosed were placed in them, provided that they were 1-2 days old and after the colonies were transferred into the tubes using a needle. The sterile tubes were shaken well with a vibrating device to homogenize the suspension. Then the density of the suspension was measured by measuring the density or turbidity, provided that it did not exceed the specified limits for the device, which is that the density of the yeasts was between 1.80 - 2.20.

After that, the cards were placed inside the tubes, and then the number data was entered Using the portable Bar Code device, laser beams were directed at the serial number of each card, and it was entered into the calculator in a special list for each template, which included ten isolates for testing only. After that, a number was written for each isolate, and the data was saved, and then the template was entered in a section called Filler. The door is empty of air, then we close the door and press the Start button. The process continues for 70 seconds, during which liquids are drawn from the tubes into the cards. When it is finished, it marks the

DOI: 10.5281/zenodo.13824711

inside of the screen with the word “Complete,” and then the mold is transferred to the second door called “Loader,” and the door is closed after three and a half minutes. The operations are completed within this section, where the device thermally cuts the capillary tube connected to each card, which is called the process (Sealing), and then the device raises the cards for the purpose of working on them inside the device, which is called the process (Loading) Then he indicates the word “Remove” and requests that the door be opened and the mold be removed. After that, the samples are incubated inside the device until the next day, when the results are completed and presented in the form of a report. (Funke, et.al. 1998). The work was in the bacteriological laboratory / Al-Sadr Teaching Hospital / Maysan Governorate.

Results

1. Diagnosis using the VITEK 2 COMPACT device

All 300 isolates were diagnosed using the Vitek 2 Compact device. For the purpose of diagnosis, 15 kits were used specifically for the diagnosis of yeasts of the YST type Vitek 2 KIT (USA). The results showed that there is a biological diversity of different types of yeasts, of their cystic and basidiophilic types, as shown in the table. Below:-

Table (1) Types of yeast isolated from water and sediments of rivers in Basra Governorate and identified using the VITEK 2 COMPACT device technology.

Number of isolates	Isolated species	the site
1	*Rhodotorula glutinis*	University of basrah
1	*R. minuta*	
2	*Cryptococcus laurentii*	
1	*Candida famata*	
1	*C. krusei*	
1	*C. lusitaniae*	
3	*C. glabrata*	
5	*C. tropicalis*	
1	*R. glutinis*	Marine Sciences Center fish tanks
1	*Cryptococcus laurentii*	
2	*C. albidus*	
1	*C. krusei*	
1	*C. guilliermondii*	
1	*C. lipolytica*	
2	*C. glabrata*	
3	*C. tropicalis*	
1	*Stephanoascus ciferrii*	Ironing bridge
1	*Cryptococcus laurentii*	
1	*C. albidus*	
1	*C. guilliermondii*	
1	*C. krusei*	
3	*C. glabrata*	
7	*C. tropicalis*	
1	*C. famata*	
1	*R. glutinis*	Al-Fayhaa
3	*C. Laurentii*	
1	*C. famata*	
1	*C. krusei*	
4	*C. glabrata*	
3	*C. tropicalis*	
1	*R. glutinis*	Sinbad
1	*Kloeckera spp.*	
2	*C. Laurentii*	
1	*C. albidus*	
1	*C. krusei*	
3	*C. glabrata*	
4	*C. tropicalis*	
1	*R. minuta*	alashar
1	*C. Laurentii*	
1	*C.albidus*	
1	*C. parapsilosis*	
3	*C. glabrata*	
4	*C. tropicalis*	
1	*S. ciferrii*	Shatt Basrha
1	*C. Laurentii*	
1	*C. parapsilosis*	
1	*C. kefyr*	
3	*C. glabrata*	
5	*C. tropicalis*	
1	*S. ciferrii*	Silk station
1	*R. glutinis*	
1	*Kloeckera spp.*	
1	*Cry. Laurentii*	
1	*C. famata*	
1	*C. parasilosis*	
5	*C. glabrata*	
3	*C. tropicalis*	

DOI: 10.5281/zenodo.13824711

1	*C. Laurentii*	Abu Al-Khasib
1	*C. albidus*	
1	*C. kefyr*	
3	*C. glabrata*	
3	*C. tropicalis*	
1	*C. famata*	Khor Abdullah
4	*C. glabrata*	
3	*C. tropicalis*	
1	*C. famata*	Khor Al-Zubair
3	*C. glabrata*	
7	*C. tropicalis*	
7	*C. glabrata*	FAO
5	*C. tropicalis*	
1	*C. Laurentii*	City
1	*C. albidus*	
1	*C. krusei*	
3	*C. tropicalis*	
1	*S. ciferrii*	The Huir
1	*C. Laurentii*	
1	*C. guilliermondii*	
2	*C. glabrata*	
4	*C. tropicalis*	
3	*C. Laurentii*	River of Ezz
1	*C. guilliermondii*	
1	*C. glabrata*	
3	*C. tropicalis*	
1	*C. famata*	
1	*R. glutinis*	aldiyr
1	*C. Laurentii*	
1	*C. parapsilosis*	
5	*C. glabrata*	
6	*C. tropicalis*	
5	*C. Laurentii*	Gourna
1	*C. albidus*	
1	*C. famata*	
3	*C. glabrata*	
4	*C. tropicalis*	
1	*S. ciferrii*	Salihiya
1	*C. Laurentii*	

1	*C. famata*	
3	*C. glabrata*	
5	*C. tropicalis*	
1	*R. glutinis*	Al-Zariji
1	*Kloeckera spp.*	
5	*C. Laurentii*	
1	*C. glabrata*	
2	*C. tropicalis*	
1	*C. Laurentii*	Silo
1	*C. famata*	
1	*C. glabrata*	
4	*C. tropicalis*	
3	*C. albidus*	Kurdlan
1	*C. famata*	
4	*C. glabrata*	
2	*C. tropicalis*	
1	*C. Laurentii*	Al-Asafiya
1	*C. parapsilosis*	
1	*C. glabrata*	
6	*C. tropicalis*	
1	*R. glutinis*	Almaeqal 1
3	*C. Laurentii*	
1	*C. famata*	
1	*C. kefyr*	
1	*C. lusitaniae*	
1	*C. lipolytica*	
8	*C. glabrata*	
5	*C. tropicalis*	
1	*C. Laurentii*	Almaeqal2
2	*C. albidus*	
1	*C. guilliermondii*	
6	*C. glabrata*	
7	*C. tropicalis*	
1	*C. Laurentii*	Ports Department
1	*C. albidus*	
1	*C. krusei*	
1	*C. lusitaniae*	
4	*C. glabrata*	
9	*C. tropicalis*	

DOI: 10.5281/zenodo.13824711

2. Calculating the percentage frequency and spatial occurrence of yeasts isolated from water and sediments:-

Spatial Frequency and Occurrence of yeast isolates

The results (Table 14) showed that the C. tropicalis type was the most frequent among the isolated species, with a frequency rate of 36.3%, while C. lipolytica recorded the lowest frequency among the isolated species, reaching 0.7%.

Table (2) Percentage frequency of types of yeast isolated from water and sediments of different rivers in Basra Governorate

Frequency ratio%	Number of isolates	Isolated species	Sequence
4	12	*Candida famata*	1
2.3	7	*C. krusei*	2
1	3	*C. lusitaniae*	3
0.7	2	*C. lipolytica*	4
1	3	*C. kefyr*	5
1.7	5	*C. parapsilosis*	6
1.7	5	*C. guilliermondii*	7
28.3	85	*C. glabrata*	8
36.3	109	*C. tropicalis*	9
12.3	37	*Cryptococcus laurentii*	10
4.7	14	*C. albidus*	11
1	3	*Kloeckera spp.*	12
2.3	7	*Rhodotorula glutinis*	13
1	3	*R. minuta*	14
1.7	5	*Stephanoascus ciferrii*	15
100	300	total summation	

As for the appearance rate of the isolated species according to the sites from which they were isolated, the results showed that the Al-Maqal 1 site showed the highest presence of isolated species, as it recorded an appearance rate of 84%, while the Medina site showed the lowest presence of isolated species, as it recorded an appearance rate of 24% (Table 15).

Table (3) Percentage spatial occurrence of yeast species isolated from water and sediments of different rivers in Basra Governorate

Appearance rate%	Number of isolates	Collection areas	Sequence
60	15	University of basrah	1
48	12	Marine Sciences Center fish tanks	2
64	16	Ironing bridge	3
52	13	Al-Fayhaa	4
52	13	Sinbad	5
44	11	alashar	6
48	12	Shatt Basrha	7
56	14	Silk station	8
36	9	Abu Al-Khasib	9
32	8	Khor Abdullah	10
44	11	Khor Al-Zubair	11
48	12	FAO	12
24	6	City	13
36	9	The Huir	14
36	9	River of Ezz	15
56	14	aldiyr	16
56	14	Gourna	17
44	11	Salihiya	18
40	10	Al-Zariji	19
28	7	Silo	20
40	10	Kurdlan	21
36	9	Al-Asafiya	22
84	21	Almaeqal1	23
68	17	Almaeqal2	24
68	17	Ports Department	25
	isolates 300	total summation	

Discussion

Yeasts are nothing but microscopic living organisms related to fungi, most of which are single-celled and non-motile. Their typical reproduction is vegetative or asexual reproduction by budding. The reason for its separation from fungi is that dealing with it in the laboratory for diagnosis is done in ways similar to the methods of diagnosing bacteria, and it consists of about 90 tests used to diagnose the phenotypic, physiological and biochemical characteristics and sexual and asexual structures of yeasts (Phaff et al., 1978 and Arx, 1980). Yeasts are distributed among basidiomycetes, cysts, and imperfect fungi (Barnett et al., 1990).

The results of the current study have proven the presence of yeasts in most rivers and sediments of those rivers from different locations in Basra Governorate / Iraq, as well as from the marine waters of that governorate. This is evidence of the ability of yeasts to grow and coexist in various environments and according to environmental factors such as salinity, temperature, acidity, etc. This is in agreement with studies by some researchers, which have proven that yeasts are microscopic organisms that prefer an acidic pH of up to 5.5. They have also been found to have an acidic pH of 2, but do not prefer a basic pH. It has also been found that they have the ability to grow in high salt concentrations in some environments (Phaff et al. al., 1978). It prefers to grow at a

DOI: 10.5281/zenodo.13824711

temperature between 15-30°C, whether in the environment or laboratory (Phaff and Starmer, 1987).

Yeasts, or yeast-like fungi, are living organisms that have the ability to exist everywhere in the aquatic ecosystem and soil, as they are organisms with the ability to tolerate salt in the aquatic environment over a wide range of salinity, temperature, oxygen level, and acidity (Boguslawska - Was and Dabrowski, 2001). Therefore, yeasts have been isolated from lakes and marine sediments (Mac Gillivray and Shia-Ris, 1993; Boguslawska-Was and Dabrowski, 2001). That is, they are present at all levels of the water column (Wurzbacher et al., 2010).

They were isolated from oligotrophic lakes in Patagonia, Argentina (Brandao et al., 2011), isolated from mesotrophic lakes in the United States (Van Uden and Ahearn, 1963), and also from lakes receiving or receiving wastes of different origins (Meyers et al., 1970), and it was isolated from recreational tourist lakes in Brazil (Medeiros et al., 2008). In all reported cases of yeast isolation, they belong to the following genera: Candida, Rhodotorula, and Cryptococcus. All of these genera are pathogenic even if they are present in polluted lakes. By the activities of humans and animals or from unpolluted lakes, it is considered biological evidence of the level of pollution that affects all aspects of life from aquatic organisms or also humans, animals and even plants, due to the attachment of living organisms to the aquatic environment (Hagler, 2006).

The study of Hagler and his group (1995) confirmed the diversity of yeasts in the ecosystem in Brazil, and the study of Silva-Bedoya and his group (2014) also confirmed the biological diversity of yeasts in the muddy sediments and water of artificial lakes in Colombia. There are many studies that agree with this study, including (Nagahama et al. .,2001; Urano et al.,2001; Kandasamy et al.,2012; Chen et al.,2009).

As for the use of the Vitek 2 compact system technology in the current study, it confirmed the accuracy and speed of diagnosis with this technology, which is used to diagnose many microscopic organisms, including yeasts, bacteria, viruses, microalgae, and parasites. This agreed with the study (Mondelli et al., 2012), which demonstrated The Vitek - Biomerieux system (Durdam, USA) is a fast and accurate technology that should be used in laboratory diagnosis of microorganisms and in all routine laboratories. Or a study (Ligozzi et al., 2002), which demonstrated the development of this technology in that it is also possible to diagnose the antigen for each pathogenic microorganism using a diagnostic card.

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DOI: 10.5281/zenodo.13824711

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DOI: 10.5281/zenodo.13824711