A Novel Report on Killer Yeast Strains Identification Methods

Article *in* Basrah Journal of Agricultural Sciences · June 2020

DOI: 10.37077/25200860.2020.32.1.04

CITATION
1

READS
89

3 authors:

Najwa Abu-Mejdad
University of Basrah
50 PUBLICATIONS **41** CITATIONS
SEE PROFILE

Adnan Al-Badran
University of Basrah
61 PUBLICATIONS **287** CITATIONS
SEE PROFILE

Abdullah Alsaadoon
University of Basrah
52 PUBLICATIONS **163** CITATIONS
SEE PROFILE

Available online at http://bjas.bajas.edu.iq
https://doi.org/10.37077/25200860.2020.32.1.04
College of Agriculture, University of Basrah

Basrah Journal of Agricultural Sciences

ISSN 1814 – 5868 Basrah J. Agric. Sci., 33(1): 39-49, 2020 E-ISSN: 2520-0860

A Novel Report on Killer Yeast Strains Identification Methods

Najwa M.J.A. Abu-Mejdad [1*], Adnan I. Al-Badran[2], Abdullah H. Al-Saadoon[3]

[1,2]Department of Biology, College of Science, University of Basrah, Basrah, Iraq.

[3]Department of Pathological Analyses, College of Science, University of Basrah, Iraq

***Corresponding author e-mail:** najwa_22_4_1978@yahoo.com
Received 19 September 2019; Accepted 31 December 2019; Available online 24 June 2019

Abstract: This study was conducted to isolate and identify killer yeasts from soil samples that collected from different locations in Basrah and Dhi-Qar provinces. Seventy-five soil samples were collected from different areas, including sandy, arable, surface sediment and uncultivated soil, using dilution methods to cultivate a serial dilution of each soil sample. The results showed that a 112 isolates were identified biochemically using VITEK system and molecularly using internal transcribed spacer (ITS1- 5.8S-ITS2) marker. The molecular identification provided fast and precise identification results for the 112 isolates, whereas the VITEK test resulted low identification efficiency (8.2% were accurate and 91.8 % were not). The Diazonium blue B salts produced a good colour reaction in distinguishing between ascomycetes and basidiomycetes. The PCR was more accurate in identification of killer yeasts compared to the VITEK system.

Key words: Diazonium blue B, VITEK, PCR, Killer yeasts, Diagnosis.

Introduction

The morphological, biochemical and molecular identification of killer yeasts have been carried out in comprehensive studies and various identifications. The killer yeasts can be grouped into three categories based on genetic determinants that encoding the killer activities that are either cytoplasmically inherited encapsulated dsRNA viruses, linear dsDNA plasmids or nuclear genes (Schmitt & Breinig, 2002). According to the molecular identification, each killer toxin has been found to possess its own novel system for toxicity .(Magliani *et al.*, 1997).

However, the morphological identification methods are not enough to be considered as a single yeast identification tool. This means that these methods require high experience due to unstable or variable characteristics. For example, the Diazonium blue B salt as a morphological test is considered good in discriminating between basidiomycetes species that showing red colour colony when reacting against this salt, and the ascomycetes yeast presented a brown colour colony (negative reaction). This salt therefore can be

considered as a good test to differentiate between the groups of fungi depending on the different colour consequences. This difference of colony colours can be attributed to different components in cell wall of ascomycetes and basidiomycetes, and also depending on experience of lab technicians (Van der Walt, 1976. , Rodrigues, 2018) Thus, the mycologists use biochemical identification to achieve accurate identification of yeast, such as commercial miniaturized systems, VITEK, API 32C and API 20C AUX from BioMerieux, which are not in common but also fast, effective and commercially available biochemical systems. When combining biochemical profiles that can be obtained by using systems such as the VITEK yeast biochemical card (YBC) (Latouche *et al.*, 1997) and the various API kits (i.e., 20C and ID 32C) to computerized databases, their use in rapid yeast identification has become more interesting tests that require less time and technical expertise compared to the morphological criteria. However, biochemical profiles can be varied due to their minor changes in test conditions. Accordingly, several reports have indicated needs of confirming identification by combining the biochemical profiles and morphological observations (Török & King, 1991). Even though, most diagnostic laboratories still rely on the conventional or traditional methods in identification of yeast species as the mainstay of species identification, but these methods are difficult, time-consuming and can lead to wrong identification. On the other hand, progress in molecular biology has provided a large number of DNA based techniques for identifying and characterizing yeasts (Hierro *et al*., 2004). Moreover, molecular techniques have become increasingly used as tools for yeast identification, especially when dealing with species that their morphological features are difficult to distinguish. The applications of these techniques have generated a greater number of studies on the classification, identification and ecology of yeast species (Tekpinar & Kalmer, 2019). This study aimed to identify yeasts using morphological, biochemical and molecular methods.

Materials & Methods

Sample collection

During this study, 75 soil samples were collected from sandy, arable, surface sediment and uncultivated soil, and the dilution method was used for fungal isolation.

Diazonium blue B stain (DBB stain)

The DBB stain was prepared from dissolving 0.1 gm of DBB salt in 100 ml of Tris-HCl buffer (0.1 M).

0.1 M Tris HCl (1 X)

The buffer was prepared by dissolving 1.211 gm of Tris HCL in 80 ml of D.W. then the volume was completed to 100 ml and the pH value was adjusted to 7.0 (Dufour *et al.*, 2003**).**

Diazonium blue B test on solid media

The yeast isolates were cultured on SDA 0.5% yeast extract, incubated for 7 days at 25 °C, then 1-2 drops of stain was added to the culture after 2-3 days of incubation. The pink or violet colour was considered as a positive, result while the brown colour was considered as a negative (Hagler & Ahearn, 1981).

Isolation of yeasts

One gram of soil from each sample was added to a sterile 15 ml test tube containing 9 ml of different solvents (peptone water 0.1 %, distilled water, phosphate buffered saline, normal saline) then mixing by vortex for 15 minutes to give 1/10 dilution 1 (D1). Serial dilution of 10^{-1}- 10^{-3} was performed, and one ml of each dilution was culture on

solid media then incubated at 25°C for 72 hours.

Biochemical identification

The biochemical identification of yeast colonies was performed by VITEK 2 system (Biomerieux, USA) using yeast biochemical card (YBC) and according to the manufacturer instructions.

Molecular study

DNA extraction

The DNA was extracted from yeast isolate according to the procedure of Presto ™ Mini gDNA Yeast Kit (Geneaid, Taiwan).

Polymerase chain reaction (PCR)

The PCR technique was used to amplify the internal transcribed spacer (ITS1- 5.8S-ITS2) region for 134 isolates using two universal primers (ITS1 and ITS4) for amplification (Bellemain *et al.*, 2010)

Primer	Primer sequences (5'- 3')	Length
ITS1	F-5-TCC GTA GGT GAA CCT GCG G-3	19 base
ITS4	R-5-TCC TCC GCT TAT TGA TAT GC-3	20 base

Sequencing of *ITS1- 5.8S - ITS2 rDNA* Gene

Sample preparation for sequencing

Twenty µl of ITS1-5.8S-ITS2 rDNA PCR product of each sample was send to Macrogen company, Korea (http://dna.macrogen.com) for sequencing.

Identification of yeasts species

The sequence results of yeasts species were analyzed using basic local alignment search tool (BLAST) to compare our sequences with deposited copies in National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov).

Results

The morphological, biochemical tests and molecular diagnosis results (Tables 1 and 2; fig. 1) revealed that the identified isolates were belonged to 112 type strains.

Diazonium Blue B (DBB) test to detect basidiomycetous yeasts

The most cells of basidiomycetous yeasts showed pink to red or violet colour reactions against DBB when using many solid growth media. However, there were great differences among isolates in the colour intensity and culture ages, which were necessary for obtaining the positive reaction. *Rhodotorula mucilaginosa* showed a positive results after twenty four hour at 25°C, whereas *Filobasidium oeirense* took three weeks to give a positive results. In contrast, some basidiomycetes got a negative results, such as *Symmetrospora folicola*, *Vishniacozyma carnescens*, *Cystobasidium benthicum* and *Cystobasidium minutum.* On the other hand, the most ascomycetous yeasts presented negative results, except *Candida membranifaciens* and *Candida glabrata* (Fig. 1, Table 1).

Table (1): DBB reaction test to distinguish between basidiomycetous yeasts and ascomycetous yeasts.

No.	Species	DBB reaction
1	*Aureobasidium melanogenum*	-
2	*Cadida tropicalis*	-
3	*C. membranifaciens*	2
4	*C. glabrata*	2
5	*Cutaneotrichosporon dermatis*	2
6	*Cystobasidium benthicum*	-
7	*C. minutum*	-
8	*Debaryomyces hansenii*	-
9	*Filobasidium oeirense*	3
10	*Galactomyces pseudocandidum*	-
11	*G. reessii*	-
12	*Geotrichum candidum*	-
13	*Hanseniaspora uvarum*	-
14	*Lodderomyces elongisporus*	-
15	*Meyerozyma caribbica*	-
16	*Naganishia adeliensis*	1
17	*N. albida*	1
18	*N. albidosimilis*	2
19	*N.diffluens*	3
20	*N. liquefaciens*	3
21	*N. uzbekistanensis*	3
22	*N. vishniacil*	2
23	*Pichia fermentans*	-
24	*Rhodotorula diobovata*	2
25	*R. mucilaginosa*	3
26	*Symmetrospora folicola*	-
27	*Torulaspora delbrueckii*	-
28	*Vishniacozyma carnescens*	-
29	*Wickerhamomyces anomalus*	-
30	*Wickerhamomyces onychis*	-
31	*Yarrowia lipolytica*	-

(Positive results): 1, Pink violet; 2, violet; 3, dark violet to purple (negative results) yellow or brown

Note: Highlighted yellow colour means basidiomycetes that gave negative results in DBB test, while highlighted green colour means ascomycetes that gave positive results in violate colour on 2 solid media as a result of DBB test.

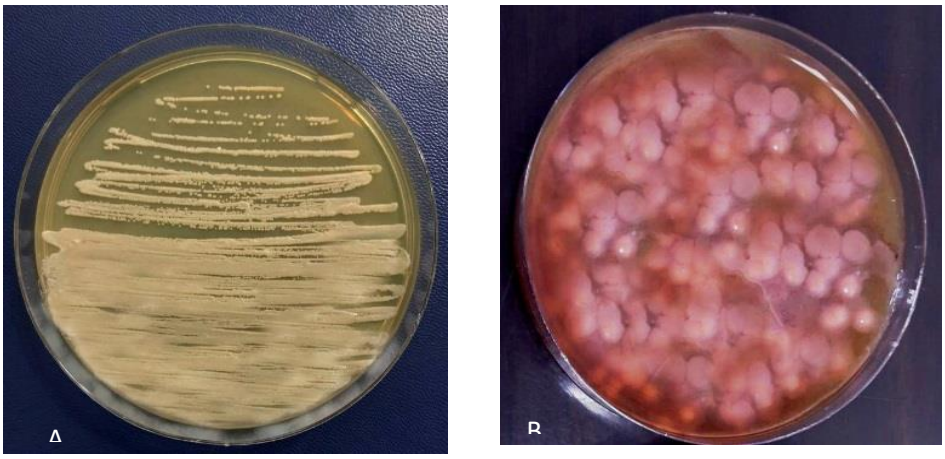

Fig. (1): DBB test (A): *Wickerhamomyces anomalus* – *ve* (B): *Rhodotorula mucilaginosa* +ve (3).

Comparison between yeast identification by VITEK 2 system and ITS1-ITS2 rDNA gene Sequencing

The comparison between VITEK and molecular identification technique (Table 2) to determine the species level of 112 yeast isolates was not identical. Only 8.2% (11 isolates, No. 11, 62, 72, 75, 160, 164, 203, 215, 234, 246 and 253) were identified as *Candida tropicalis* by both techniques, while the rest (91.8%) were completely mismatched.

Table (2): A comparison between VITEK and *ITS1-ITS2 rDNA gene* identification.

Isolates	Species name in gene bank	Homology	VITEK	Identification
102	*Aureobasidium melanogenum*	100%	Un-identified	
87	*Candida membranifaciens*	100%	Un-identified	
170	*C. membranifaciens*	100%	Un-identified	
236	*C. membranifaciens*	100%	Un-identified	
11	*C. tropicalis*	100%	*Candida tropicalis*	96%
62	*C. tropicalis*	100%	*C. tropicalis*	96%
72	*C. tropicalis*	100%	*C. tropicalis*	96%
75	*C. tropicalis*	100%	*C. tropicalis*	96%
158	*C. tropicalis*	100%	*C. tropicalis*	96%
164	*C. tropicalis*	100%	*C. tropicalis*	96%
203	*C. tropicalis*	100%	*C. tropicalis*	96%
215	*C. tropicalis*	100%	*C. tropicalis*	96%
234	*C. tropicalis*	100%	*C. tropicalis*	96%
246	*C. tropicalis*	100%	*C. tropicalis*	96%
253	*C. tropicalis*	100%	*C. tropicalis*	96%
160	*C. glabrata*	100%	*C. tropicalis*	94%
49	*C. membranifaciens*	100%	Un-identified	
173	*C. membranifaciens*	100%	Un-identified	
188	*Cryptococcus albidisimilis*	*99%*	*Cryptococcus*	97%
134	*C. diffluens*	100%	*Cryptococcus*	93%
218	*C. uzbekistanensis*	*99%*	Un-identified	
185	*C. albidus*	100%	*C. laurentii*	99%

169	*Cutaneotrichosporon dermatis*	100%	*Trichosporon asahii*	88%
36	*Cystobasidium benthicum*	*99%*	Un-identified	
71	*C. benthicum*	100%	Un-identified	
152	*C. benthicum*	99%	Un-identified	
18	*C. minutum*	*98%*	*Un-identified*	
81	*Debaryomyces hansenii*	100%	*Candida famata*	99%
132	*D. hansenii*	100%	*C. famata*	99%
193	*D. hansenii*	100%	*C. famata*	99%
201	*D. hansenii*	100%	*C. famata*	99%
208	*D. hansenii*	100%	*C. famata*	99%
235	*D. hansenii*	100%	*C. famata*	99%
255	*D. hansenii*	100%	*C. famata*	99%
256	*D. hansenii*	100%	*C. famata*	99%
262	*D. hansenii*	100%	*Candida lipolytica*	92%
80	*Filobasidium oeirense*	100%	*C. laurentii*	91%
28	*Galactomyces pseudocandidum*	100%	*C. famata*	95%
63	*Galactomyces reessii*	100%	*C. famata*	95%
70	*Geotrichum candidum*	100%	*C. famata*	95%
163	*G. candidum*	100%	*C. famata*	95%
78	*Hanseniaspora uvarum*	100%	Un-identified	
213	*H. uvarum*	100%	Un-identified	
2	*Lodderomyces elongisporus*	100%	*C. famata*	95%
136	*L. elongisporus*	100%	*C. famata*	95%
46	*Meyerozyma caribbica*	100%	Un-identified	
47	*M. caribbica*	100%	Un-identified	
55	*M. caribbica*	100%	Un-identified	
124	*Naganishia adeliensis*	*99%*	*C. famata*	95%
108	*N. albida*	100%	*C. albidus*	96%
180	*N. albida*	100%	*C. laurentii*	95%
241	*N. albida*	100%	*C. albidus*	96%
129	*N. albidosimilis*	*99%*	*C. laurentii*	97%
65	*N. diffluens*	100%	*C. albidus*	93%
116	*N. diffluens*	100%	*C. laurentii*	95%
138	*N. diffluens*	100%	*C. laurentii*	
198	*N. diffluens*	100%	*C. albidus*	93%
261	*N. diffluens*	100%	*C. albidus*	93%
204	*N. diffluens*	100%	*C. albidus*	93%
69	*N. liquefaciens*	100%	*C. laurentii*	91%
137	*N. liquefaciens*	100%	Un-identified	
189	*N. vishniacil*	100%	*C. albidus*	91%
154	*Pichia fermentans*	99%	*Candida krusei*	93%
195	*P. fermentans*	100%	*C. krusei*	94%
200	*P. fermentans*	99%	*C. krusei*	93%
3	*Rhodotorula diobovata*	100%	*C. laurentii*	94%
53	*R. diobovata*	100%	*C. laurentii*	94%
141	*R. diobovata*	100%	*C. laurentii*	94%
33	*R. mucilaginosa*	100%	*C. albidus*	93%
82	*R. mucilaginosa*	100%	*C. albidus*	93%
111	*R. mucilaginosa*	100%	*C. albidus*	93%
139	*R. mucilaginosa*	*99%*	*C. albidus*	93%

144	*R. mucilaginosa*	100%	*C. albidus*	93%
148	*R. mucilaginosa*	100%	*C. albidus*	93%
159	*R. mucilaginosa*	100%	*C. albidus*	93%
187	*R. mucilaginosa*	100%	*C. albidus*	93%
194	*R. mucilaginosa*	100%	*C. albidus*	93%
196	*R. mucilaginosa*	100%	*C. albidus*	93%
210	*R. mucilaginosa*	100%	*C. albidus*	93%
216	*R. mucilaginosa*	100%	*C. albidus*	93%
237	*R. mucilaginosa*	100%	*C. albidus*	93%
245	*R. mucilaginosa*	100%	*Rhodotorula glutinis*	94%
265	*R. mucilaginosa*	100%	*C. laurentii*	95%
219	*Symmetrospora folicola*	100%	Un-identified	
174	*Torulaspora delbrueckii*	100%	Un-identified	
177	*T. delbrueckii*	100%	Un-identified	
270	*T. delbrueckii*	100%	Un-identified	
105	*Vishniacozyma carnescens*	100%	Un-identified	
224	*Wickerhamomyces anomalus*	100%	Un-identified	
155	*W. onychis*	100%	Un-identified	
1	*Yarrowia lipolytica*	100%	*Malassezia furfur*	97%
26	*Y. lipolytica*	100%	*M. furfur*	97%
29	*Y. lipolytica*	100%	*Candida*	97%
90	*Y. lipolytica*	100%	*M. furfur*	97%
92	*Y. lipolytica*	100%	*M. furfur*	97%
100	*Y. lipolytica*	100%	*M. furfur*	97%
110	*Y. lipolytica*	100%	*M. furfur*	97%
112	*Y. lipolytica*	100%	*M. furfur*	97%
147	*Y. lipolytica*	100%	*M. furfur*	97%
165	*Y. lipolytica*	100%	*M. furfur*	97%
176	*Y. lipolytica*	100%	*M. furfur*	97%
222	*Y. lipolytica*	100%	*M. furfur*	97%
227	*Y. lipolytica*	100%	*M. furfur*	97%
230	*Y. lipolytica*	100%	*M. furfur*	97%
238	*Y. lipolytica*	100%	*M. furfur*	97%
243	*Y. lipolytica*	100%	*M. furfur*	97%
244	*Y. lipolytica*	100%	*M. furfur*	97%%
248	*Y. lipolytica*	100%	*M. furfur*	97%
259	*Y. lipolytica*	100%	*M. furfur*	97%
263	*Y. lipolytica*	100%	*M. furfur*	97%
264	*Y. lipolytica*	100%	*M. furfur*	97%
266	*Y. lipolytica*	100%	*M. furfur*	97%

Discussion

Diazonium Blue B (DBB) test to detect basidiomycetes yeasts on solid media

The DBB test has been used by yeast taxonomists to distinguish between ascomycetous and basidiomycetous fungi. During the current study, the most cells of basidiomycetous yeasts showed pink to red or violet colour reactions (+) against DBB, even when using many solid growth media, while

ascomycetes yeast presented brown colour (-) results.

Robert *et al.* (2015) stated that the DBB colour reaction (reagent) is used as a primary assistant factor in differentiation between basidiomycetous (especially those with unknown teleomorph) and ascomycetous yeasts.

This test is influenced by four factors (temperature of reagent, type of culture medium that used with the reagent, incubation period of tested yeast and pH of culture media, which are used during the test). However, an experienced technician will implements this test more advantageous than other workers who performs a routine identification (Kreger-van Rij & Veenhuis, 1971). A neutral pH (≈7.0) of medium was more crucial for a positive DBB colour reaction (Singh & Kaur, 2018). There was considerable variation between isolates in their colour intensity (graduated from orange, pink and red) and the age of culture, which was necessary for obtaining a positive reaction (Rodrigues & Fonseca, 2003). In our findings, most environmental yeasts were required incubation period from 7-14 days to give positive result occurrence, while a few genera like *R. mucilignosa* and *W. anomalus* showed a positive results after 24 h.

VITEK 2 compact system

The VITEK biochemical procedures of yeasts (YBC) led to quick identification, especially for clinical specimens. Moreover, these methods are still helpful for primary identification of ecological specimens. During this study, a total of 112 yeast isolates were tested by VITEK 2 compact system but only 8.2% of them were identified accurately, whereas, 91.8% of them were not. According to this high percentages of improper identification of many isolates, it can be clearly stated that, this test is unreliable for ecological yeasts compared to the clinical isolates, especially for common yeasts. This finding is correspondent with the results of Westblade *et al.* (2013). Anyhow, the VITEK yeast biochemical system may be used to identify the common yeasts i.e., *Candida tropicalis, C. parapsilosis, Candida glabrata, C. albicans* and *Cryptococcus neoformans* with a period of twenty-four hours (93% of precise identifications). The ability of the YBC system to determine these common isolates quickly due to an automated system, which is used in several clinical laboratories (Zhao *et al.*, 2017). Our results confirmed that the repeated improper identification for many isolates *i.e. Yarrowia lipolytica, Debaryomyces hansenii, Candida tropicalis, Rhodotorula mucilignosa* etc. were in accordance with the findings of Dooley *et al.* (1994). Delayed biochemical reactions in VITEK system suggested that unreliable confirmation for the YBC and validation of identification by molecular technique (Valenza *et al.*, 2008). In addition, the YBC may result in improper identification due to delayed growth of yeasts (more thanseven days), especially where workers in microbiological laboratories have less professional mycological expertise and did not give enough time to yeasts to grow before starting the biochemical test. Similar explanation was reported by Freydiere *et al.* (2001). Furthermore, differences among these studies, may be attributed to the laboratory conditions (in equipment, reagents, or personnel skill, old version of ID card of VITEK 2 compact system) that also can lead to misidentification .

Genetic identification

Yeast identification by traditional (morphological and biochemical) methods is a complex, difficult and time-consuming

process that requires the high expertise and skills of the people concerned in this area. Sometimes, they give wrong or confused and incomplete identification results, such as identification to levels less than species. Khattab *et al.* (2016) reported that there is a need to use 90 chemical tests to bring the yeast species closer to the precise identification. In recent decades, researchers have been using molecular identification methods, which are characterized by their accuracy and rapidity in these methods, yeast strains can be isolated from substrate and identified to the species level (Alsohaili & Bani-Hasan, 2018).

Franco-Duarte *et al.* (2019) reported that polyphasic approach involves multiple identification methods, including phenotypic, biochemical and genetic approaches, which are altogether can provide a comprehensive and accurate identification.

Conclusion

The Diazonium blue B was an excellent stain for distinguishing between ascomycetous and basidiomycetous yeast. While, the VITEK 2 compact system is not recommended for the identification of ecological yeast strains in comparison to the molecular technique that was perfect.

Acknowledgements

This work was performed by the help of cell research centre, Department of Biology, College of Science, University of Basrah for providing the equipment we are gratefully acknowledged.

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

All applicable institutional, national and international guidelines for the care and use of animals were followed.

References

Alsohaili, S.A. & Bani-Hasan, B.M. (2018). Morphological and molecular identification of fungi isolated from different environmental sources in the Northern Ebvastern Desert of Jordan. Jordan J. Biol. Sci., 11(3): 329-337. http://jjbs.hu.edu.jo/vol11.htm

Bellemain, E.; Carlsen, T.; Brochmann, C.; Coissac, E.; Taberlet, P. & Kauserud, H. (2010). ITS as an environmental DNA barcode for fungi: an in silico approach reveals potential PCR biases. BMC Microbiology, 10(1): 189. https://doi.org/10.1186/1471-2180-10-189

Dooley, D.; Beckius, M.L. & Jeffrey, B.S. (1994). Misidentification of clinical yeast isolates by using the updated Vitek Yeast Biochemical Card. J. Clin. Microbiol., 32(12): 2889-2892. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC264196/

Dufour, J.; Verstrepen, K.; Derdelinckx, G., Boekhout, T. & Robert, V. (2003). Yeasts in Food-Beneficial and Detrimental Aspects: Woodhead Publishing: New York: 512pp.

Franco-Duarte, R.; Černáková, L.; Kadam, S.; S. Kaushik, K.; Salehi, B.; Bevilacqua, A. & Leszczewicz, M. (2019). Advances in chemical and biological methods to identify microorganisms-from past to present. Microorganisms, *7*(5) :1-130. https://doi.org/10.3390/microorganisms7050130

Freydiere, A.-M.; Guinet, R. & Boiron, P. (2001). Yeast identification in the clinical microbiology laboratory: Phenotypical methods. Sabouraudia, 39(1): 9-33. https://doi.org/10.1080/mmy.39.1.9.33

Hagler, A.N. & Ahearn, D. (1981). Rapid diazonium blue B test to detect basidiomycetous yeasts. Int. J. Syst. Evol. Microbiol.,, 31(2): 204-208. https://doi.org/10.1099/00207713-31-2-204

Hierro, N.; Gonzalez, A.; Mas, A. & Guillamón, J. (2004). New PCR-based methods for yeast identification. J. Appl. Microbiol., 97(4): 792-801. https://doi.org/10.1111/j.1365-2672.2004.02369.x

Khattab, S.M.R.; Abdel-Hadi, A.M.; Abo-Dahab, N.F. & Atta, O.M. (2016). Isolation, characterization, and identification of yeasts Associated with foods from Assiut City, Egypt. British Microbiol. Res. J., 13(1) :1-10. https://doi.org/10.9734/BMRJ/2016/24170

Kreger-van Rij, N. & Veenhuis, M. (1971). A comparative study of the cell wall structure of basidiomycetous and related yeasts. Microbiology, 68(1): 87-95. https://www.microbiologyresearch.org/content/journal/micro/10.1099/00221287-68-1-87;jsessionid=XzcjctX3N0rMq4P2m1gdKUIU.mbslive-10-240-10-108

Latouche, G.N.; Daniel, H.; Lee, O.C.; Mitchell, T.G.; Sorrell, T.C. & Meyer, W. (1997). Comparison of use of phenotypic and genotypic characteristics for identification of species of the anamorph genus Candida and related teleomorph yeast species. J. Clin. Microbiol., 35(12): 3171-3180. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC230143/

Magliani, W.; Conti, S.; Gerloni, M.; Bertolotti, D. & Polonelli, L. (1997). Yeast killer systems. Clin. Microbiol. Rev., 10(3): 369-400. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC172926/

Robert, V.; Cardinali, G. & Casadevall, A. (2015). Distribution and impact of yeast thermal tolerance permissive for mammalian infection. BMC Biol., 13(1): 1-18 https://doi.org/10.1186/s12915-015-0127-3

Rodrigues, M.G., & Fonseca, A. (2003). Molecular systematics of the dimorphic ascomycete genus Taphrina. Int. J. Syst. Evol. Microbiol., 53(2): 607-616. https://doi.org/10.1099/ijs.0.02437-0

Rodrigues, A., Pinheiro, R., Costa, J., Santos, J., Poli, J., Rosa, C. & Nóbrega, M. (2018). Comparison between morphophysiological and molecular methods for the identification of yeasts isolated from honey. Int. Food Res. J.l, 25(1):418-422. https://docplayer.net/103197739-Comparison-between-morphophysiological-and-molecular-methods-for-the-identification-of-yeasts-isolated-from-honey.html

Schmitt, M.J. & Breinig, F. (2002). The viral killer system in yeast: from molecular biology to application. FEMS Microbiol. Rev., 26(3): 257-276. https://doi.org/10.1111/j.1574-6976.2002.tb00614.x

Singh, R. & Kaur, N. (2018). Biochemical and molecular characterization of a new pullulan producer Rhodosporidium paludigenum PUPY-06. J. Appl. Biol. Biotechnol.,6(1):28-37. https://doi.org/10.7324/JABB.2018.60106

Tekpinar, A.D. & Kalmer, A. (2019). Utility of various molecular markers in fungal identification and phylogeny. Nova Hedwigia, 108(3-4): 3-4. https://doi.org/ 10.1127/nova_hedwigia/2019/0528

Török, T. & King, A. (1991). Comparative study on the identification of food-borne yeasts. Appl. Environ. Microbiol., 57(4): 1207-1212. https://www.ncbi.nlm.nih.gov/ pmc/articles/PMC182869/

Valenza, G.; Strasen, J.; Schäfer, F., Frosch, M., Kurzai, O., & Abele-Horn, M. (2008). Evaluation of new colourcolourimetric Vitek 2 yeast identification card by use of different source media. J. Clin. Microbiol., 46(11): 3784-3787. https://doi.org/10.1128/JCM.01318-08

Van der Walt, J. & Hopsu-Havu, V. (1976). A colour reaction for the differentiation of ascomycetous and hemibasidiomycetous yeasts. Anton. Leeuw., 42(1-2): 157-163. https://doi.org/10.1007/BF00399460

Westblade, L.F.; Jennemann, R.; Branda, J.A.; Bythrow, M.,;Ferraro, M.J.; Garner, O.B. & Mochon, A.B. (2013). Multicenter study evaluating the Vitek MS system for identification of medically important yeasts. J. Clin. Microbiol., 51(7): 2267-2272. https://doi.org/10.1128/JCM.00680-13

Zhao, Y.; Tsang, C.; Xiao, M.; Chan, J.F.; Lau, S.; Kong, F. & Woo, P.C. (2017). Yeast identification by sequencing, biochemical kits, MALDI–TOF MS and rep-PCR DNA fingerprinting. Med. Mycol., 56(7): 816-827. https://doi.org/10.1093/mmy/myx118.

تقرير جديد عن طرق تحديد سلالات الخميرة القاتلة

نجوى محمد جميل علي أبو مجداد [1] وعدنان عيسى البدران [1] وعبد الله حمود السعدون [2]

[1]قسم علوم الحياة، كلية العلوم، جامعة البصرة، العراق

[2]قسم التحليلات المرضية، كلية العلوم، جامعة البصرة، العراق

المستخلص: اجريت هذه الدراسة لعزل وتحديد الخمائر القاتلة من عينات التربة التي جمعت من مواقع مختلفة في محافظتي البصرة وذي قار. تم جمع خمسة وسبعين عينة من التربة من مناطق مختلفة ، بما في ذلك الترب الرملية والزراعية والترب الغير مزروعة ، وذلك باستخدام طرق التخفيف لزراعة كل عينة من التربة. أوضحت النتائج أنه تم تحديد 112 عزلة بطرائق كيموحيوية باستخدام نظام VITEK وجزيئيًا باستخدامنطقة الـ .(ITS1- 5.8S-ITS2) اظهرالتشخيص الجزيئي نتائج تحديد سريعة ودقيقة للعزلات 112 ، في حين أدى اختبار VITEK إلى كفاءة تحديد منخفضة (8.2٪ كانت دقيقة و 91.8٪ لم تكن كذلك). اظهرت نتائج اختبار أملاح ديازونيوم الزرقاء B تفاعلًا جيدًا للألوان في التمييز بين الفطريات الكيسية والبازيديةو كان تشخيص تفاعل البلمرة التسلسلي أكثر دقة في تحديد الخمائر القاتلة مقارنة بنظام.VITEK.

الكلمات المفتاحية: املاح الديازونيم الزرقاء **B** ,الفايتك, تفاعل البلمرة التسلسلي,تشخيص الخمائر القاتلة .