British Microbiology Research Journal
13(1): 1-10, 2016, Article no.BMRJ.24170
ISSN: 2231-0886, NLM ID: 101608140

SCIENCEDOMAIN *international*
www.sciencedomain.org

Isolation, Characterization, and Identification of Yeasts Associated with Foods from Assiut City, Egypt

Sadat Mohammad Rezq Khattab[1*], Ahmed Mustafa Abdel-Hadi[1,2], Nageh Fathy Abo-Dahab[1] and Omar Mohammad Atta[1]

[1]Department of Botany and Microbiology, Faculty of Science, Al-Azhar University, Assiut, Branch, Assiut, 71524, Egypt.
[2]Department of Medical Laboratories, College of Applied Medical Sciences, Majmaah University, 11925, Kingdom of Saudi Arabia.

Authors' contributions

Author SMRK designed the study, wrote the protocol, molecular identify yeasts isolates, revised the manuscript and submit it. Authors AMAH and NFAD wrote the protocol, supervised and revised the manuscript. Author OMA carried out the experiments and write the first draft of the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BMRJ/2016/24170
Editor(s):
(1) Marcin Lukaszewicz, Department of Biotransformation, Faculty of Biotechnology, University of Wroclaw, Wroclaw, Poland and Division of Chemistry and Technology Fuels, Wroclaw University of Technology, Wroclaw, Poland.
Reviewers:
(1) Mauricio Martins, UFSC, Brazil.
(2) Charu Gupta, Amity University, UP, India.
(3) S. S. Kanwar, Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya, Palampur, India.
Complete Peer review History: http://sciencedomain.org/review-history/13348

Original Research Article

Received 6th January 2016
Accepted 5th February 2016
Published 18th February 2016

ABSTRACT

The objective of this work was to isolate, characterize physiologically, and identify yeasts associated with foods from Assiut city, Egypt. Fifty-two colonies of yeasts associated with orange, mandarin, tomato, squash, sobia drink, mango juice, sugarcane juice, yogurt and buttermilk samples, collected from Assiut City, Egypt, were isolated. Out of which, Eleven isolates were selected randomly and subjected to morphological, biochemical studies and molecular identification techniques employing sequence of internal transcribed spacer (ITS) regions and partial D1/D2 large-subunit domains of the 26S ribosomal RNA. Identified yeasts were belonged to six genera and species; four species belonged to ascomycetes: *Debaryomyces hansenii* (five isolates), *Saccharomyces cerevisiae* (one isolate), *Candida tropicalis* (one isolate), and *Pichia kudriavzevii*

**Corresponding author: E-mail: sadatkhattab2004@yahoo.com;*

(one isolate). In addition, two yeasts species belonged to basidiomyces: *Rhodotorula mucilaginosa* (two isolates), and *Trichosporon dulcitum* (one isolate). In spite of low frequency of yeasts isolates on the tested food, mango juice and buttermilk showed the higher sources for incidences during this study.

Keywords: Yeasts isolation; Identification; milk products; fruits; juices; Assiut City, Egypt.

1. INTRODUCTION

Yeasts are unicellular eukaryotes that belong to the Kingdom of Fungi and play various roles in affecting the quality and safety of food products. They are ubiquitous, and commonly spoilage fruits, vegetables and other plant materials, in addition to, an association with soil and insects [1]. Decaying fruits are an important microhabitat for several yeast species [2]. Colonization of fast growing, fermentative, low assimilative profiles of apiculate yeasts: (*Kloeckera*, *Hanseniaspora*, and *Saccharomycodes*) initiate severe deterioration of fruits [3]. Oranges and mangoes usually rotted by spoilage of a wide variety of fermentative or weakly non-fermenting yeasts [4,5]. Basidiomycetous genera such as *Cryptococcus* and *Sporobolomyces* yeasts were dominated on leaves of mango trees [6]. In addition, ascomycetous yeasts associated the wounded parts of the phylloplane [7].

Fruit juices and soft drinks constitute suitable environment for growth of most microorganisms. Actually, beverages are excellent substrates for supporting the growth of yeasts, where the highest amount of nitrogenous compounds and vitamins promote occurrence of yeasts [8,9]. The dairy products are especially favorable environments for the growth of yeasts, where *Candida* (*C. sphaerica*) *Debaryomyces, Mycoderma, Rhodotorula,* and *Saccharomyces* (*S. dairensis* and *S. unisporus*) are the most common isolated genera in these environments [10]. Other yeasts species like *Issatchenkia orientalis*, *C. albicans*, *Clavispora lusitaniae* (*Candida lusitaniae*), *Kodamaea ohmeri* (*Pichia ohmeri*), *Kluyveromyces marxianus*, and *C. catenulate* were associated with traditional Egyptian dairy products and kariesh cheese were found to be the most diverse in its yeast floras [11]. Similarly, the most prevalent isolates in Egyptian karish cheese were belonged to *Trichosporon cutaneum* (25%), *C. catenulata* (23%), *Yarrowia lipolytica* (13%), *Debaryomyces hansenii* (13%), *Kluyveromyces lactis* (6%), *Geotrichum candidum* (7%), *C. zeylanoides* (5%), *C. lambica* (3%), *C. albicans* (2%), *Cryptococcus formans* (1%), *Rhodotorula glabrata* (1%) and *S. cerevisiae* (1%) [12]. Generally, yeasts are important microflora of many food products due to their ability to grow on a substrate rich by proteins, lipids, sugars, and organic acids [13].

Molecular identification techniques employing sequencing the internal transcribed spacer (ITS) regions and partial D1/D2 large-subunit domains of the 26S ribosomal RNA (rRNA) have become the most frequently and convenient method for identification of yeasts isolates. This study aimed to isolate yeasts associated with milk products, fruits, and juices from Assiut city, Egypt. The yeasts were identified morphologically, physiologically as well as by molecular techniques.

2. MATERIALS AND METHODS

2.1 Samples and Isolation

Three samples from each food source; orange, mandarin, tomato, squash, sobia drink, mango juice, sugarcane juice, yogurt, and buttermilk were collected from the local markets in Assiut city, Egypt. The isolation was achieved according to Lodder and Kreger [14], and Kurtzman et al. [15], where the samples were settled at room temperature (25~30ºC) for 48 hours (h), then used for isolation. Liquid samples were direct spread and streaked on yeast peptone dextrose agar (YPD), as well of a 1/10 w/v of solid samples/ sterile water were homogenized and used for direct plating techniques during this study. A medium composed of 10 g/L of yeast extract, 20 g/L of peptone, 20 g/L of dextrose, 20 g/L of agar and added ampicillin (100 mg/L), and chloramphenicol (100 mg/L) to prevent bacterial contamination, and then the plates were incubated at 28±2ºC for 10 days. Purification of yeast colonies were achieved by streaked methods. The cell/colony morphologies of the purified yeasts isolates were investigated and monitored under microscope. Yeast cultures were maintained on 2% YPD slants at 4ºC for short period storage. In addition, equal volumes of propagated yeast cultures and 80% glycerol were mixed well and stored at 80ºC for long time preservation.

2.2 Identification of Yeast Strains

Selected yeast isolates were identified based on their morphological, biochemical properties according to Kurtzman et al. [15]; Walt and Yarrow [16] While, molecular techniques according to Nisiotou et al. [17].

2.2.1 Morphological characteristics

The strains were first checked for their morphological aspects and growth patterns on solid and liquid media. Characteristic features of textures (mucoid, fluid or viscous, butyrous); elevation (flat or raised); color (yellow, orange and red); surface (glistening or dull, smooth, rough, and sectored) and margin (entire, undulating, lobed, and filaments) were investigated. The cells of a young actively growing culture from 2~3 days at 25℃ were stained by lacto phenol-cotton blue and investigated microscopically to determine the shape of cells, budding (Mono, Bi, and multipolar) or fission and conidial formation. Moreover, the formation of compact, coherent, flocculent, or mucoid sediment, a ring, floating islets or a pellicle of yeasts was examined. Additionally, formation of germ tubes was checked using 24 h cultivated yeasts and incubated at 37℃ for 2~3 h in blood serum then investigated under light microscope according to Kurtzman et al. [15].

2.2.2 Physiological and biochemical characteristics

The ability of eleven yeasts isolates to utilize and grow aerobically on carbon and nitrogen as a sole source of energy was studied. Several carbon sources (D-glucose, D-galactose, lactose, maltose, sucrose, raffinose, soluble starch, D-ribose, L-rhamnose, D-xylose, glycerol, D-mannitol, ethanol, and methanol) used in this study. While the nitrogen sources used were nitrate, nitrite, and L-lysine. Assimilation tests were achieved by replica plate method, where a set of plates contained different carbon or nitrogen source in a carbon or nitrogen basal agar medium were inoculated by yeasts isolates. The growth of colonies of negative control plates (without carbon or nitrogen sources) was compared with plates supplemented with carbon or nitrogen sources after 24~48 h of incubation [15].

On the other hand, fermentation abilities of yeast isolate to ferment 2% sugar solutions of (glucose, lactose, maltose, raffinose, galactose, sucrose, and xylose) and 4% of raffinose was tested. Pure yeast isolates were cultivated on YPD liquid medium for 48 h and then the cell pellets were collected by centrifugation. Fermentation was started by addition of sugar solutions to cell pellets and resuspended prior to insert Durham tubes (inverted without any air in aseptic condition) to collect any gas produced during fermentation. Thereafter, the tubes were incubated at 25℃ for up to 28 days. The fermentation abilities were inspected at frequent intervals for accumulated gas in the Durham tubes, as well as changes in indicator (phenol red) color were investigated according to Kurtzman et al. [15].

Moreover, other physiological characteristics were performed to examine the osmotic pressure of yeast isolates such as growth abilities on 50, 60% w/v glucose, and 10% NaCl plus 5% glucose. In addition to, the ability of growth at 37℃, urea hydrolysis, starch formation, tolerance to 1% acetic acid. Furthermore, acid production from glucose of Custer's chalk medium at 25℃ after incubation of two weeks was carried out according to the methods of Kurtzman and Fell [18].

2.2.3 Molecular identification

Purified yeast isolates were identified based on sequences of rRNA gene of large subunit (LSU) according to Nisiotou et al. [17] as the following: Genomic DNA was extracted from overnight grown yeast cells in broth YPD medium at 28℃ and 150 rpm. Yeasts cells pellets were collected by centrifugation and resuspended in a lysis buffer (2% Triton X-100, 1% sodium dodecyl sulfate, 100 mM NaCl, 10 mM Tris-HCl pH 8.0, and 1 mM EDTA) with glass beads and mixture of phenol, chloroform, isoamyl alcohol (25:24:1). Thereafter, vigorously vortex the mixture to lysis yeast cell walls and DNA precipitated by chilled absolute alcohol as described previously [19].

PCR thermal cycler started amplification of rDNA in 50 µL reaction mixture, containing 5 µL 10X KOD buffer, 5 µL dNTP mixtures, 3 µL $MgSO_4$, 15 pmol of each primer, 1 µL KOD polymerase and 100 ng of genomic DNA. PCR conditioned to denaturation at 94℃ for 10 s, annealing at 50℃ for 30 s, and extension at 68℃ for 1.5 min /1kbp. Amplification of the ITS1, 5.8S-ITS2, and D1/D2 domains rDNA region was achieved by forward primer ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and reverse primer NL-4 (5'-

GGTCCGTGTTTCAAGACGG-3'). PCR product was purified, dyed and sequenced as described previously [20]. Sequencing data were submitted to NCBI/BLAST (blast.ncbi.nlm.nih.gov/BLAST.cgi) for sequence alignment, to construct a phylogenetic tree, and to assign an accession numbers for the selected yeasts isolates.

3. RESULTS

3.1 Isolation Incidences and Sources of Isolates

Fifty-two yeast colonies were isolated from twenty-seven samples (nine sources) on YPD agar medium. A total of 52 isolates were obtained: yogurt (N=6), orange (N=3), tomato (N=7), squash (N=2), mandarin (N=2), mango juice (N=11), buttermilk (N=10), and Egyptian sobia drink (N=4). The isolates subjected to further streaking until completely purified then eleven isolates were selected for further studies of characterizations and identification.

3.2 Morphological Characterization

The morphological characters of selected yeast isolates were summarized in Table 1.

3.3 Biochemical Characterization

3.3.1 Assimilation tests

All selected yeast isolates assimilated glucose and maltose and were unable to assimilate methanol as a sole carbon source. Only isolate Y.5 well-assimilated citrate and xylose as reflected in the results shown in Table 2. Isolates Y.5 and Y.16 negatively and poorly assimilated sucrose, respectively, while the other isolates showed a well assimilation. Lactose efficiently assimilated by Y.7~10, 13 and 16 whereas the rest was not. For starch, only Y.5 strongly assimilated it from among the eleven-tested yeasts isolates while, Y.7~10 and 13 were weakly dissociation abilities (Table 2).

3.3.2 Fermentation tests

Seven sugars (glucose, lactose, maltose, raffinose, galactose, sucrose, D-xylose) were used during study fermentation abilities of yeasts isolates. Isolate Y.2 was able to ferment all sugars except xylose and lactose, while Y. 5 and 6 fermented only glucose. On the other hand, Y.14, 15 and 16 were unable to ferment any sugar. Yeasts isolates Y.7~10 and 13 were efficient to ferment glucose and less for galactose, while unable to ferment the rest of sugars (Table 3). The results of additional physiological characteristics were summarized in Table 4.

3.4 Molecular Identification

Eleven yeasts isolates were identified, based on molecular identification techniques using amplified fragments of ITS and D1/D2 domains of large subunit, which belonged to six genus: *D. hansenii* (five isolates), *S. cerevisiae* (one isolate), *C. tropicalis* (one isolate), *Pichia kudriavzevii* (one isolate), *R. mucilaginosa* (two isolates), and *Trichosporon dulcitum* (one isolate). In the phylogenetic tree, all strains

Table 1. Morphological characterization of yeast isolates

Isolate no.	Colony on solid medium	Cells shape	Growth in broth media
Y.2	Smooth surface, raised, cream-color	Globose, ovoidal, multilateral budding	White precipitate
Y.5	White, smooth, butyrous, and soft	Subglobose to ovoidal- single cells, and in pairs.	Rings
Y.6	Butyrous and light cream colored with small lobes	Ovoidal to elongate	Pellicles
Y.7~10 and 13	Smooth surface, raised, entire margin, white color	Spheroidal, short chains, multilateral budding	Pellicles
Y.14 and 15	Smooth surface, flat, entire margin, pink- to deep red color	Ovoidal, multilateral budding	Pink ring , precipitate
Y.16	Folded, rough surface, flat, with volcano-shaped, fringed with filaments, yellowish, pale cream-colored	Hyphae disarticulating into arthroconidia	Pellicles and rings

clustered with their nearest phylogenetic neighbors strains (Fig. 1). Their accession numbers were coordinated with identified names and listed in Table 5 and Fig. 1.

4. DISCUSSION

Isolation of yeasts from natural resources is the most successful technique to obtain yeast isolates that have abilities to utilize and ferment various exogenous compounds [21]. Isolation of yeast species associated with different food samples collected from Assiut city, Egypt, was investigated. The species *S. cerevisiae, C. tropicalis, P. kudriavzevii, D. hansenii, R. mucilaginosa, T. dulcitum* were isolated. The results showed that all these species were found in lower frequency. This could be due to that normal yeast flora on fresh, undamaged fruits are generally low, presence of preservatives for juices, and presence of lactic acid bacteria for milk products. *D. hansenii* was associated only with fruit samples and mango juice, whereas *R. mucilaginosa* was isolated only from mango juice.

Table 2. Assimilation of carbon sources using selected yeasts isolates

Isolate no.	Glucose	Galactose	L-rhamnose	D-ribose	D-xylose	Maltose	Lactose	Sucrose	Raffinose	Starch	Citrate	D-mannitol	Ethanol	Glycerol	Methanol
Y.2	+	s	-	-	-	+	-	+	w	-	-	-	w	-	-
Y.5	+	+	-	-	+	+	-	-	-	+	+	+	w	W	-
Y.6	+	w	-	-	-	w	-	+	-	-	-	-	+	+	-
Y.7	+	+	s	w	w	+	+	+	+	w	w	s	w	+	-
Y.8	+	s	+	w	w	s	s	+	+	w	w	w	w	W	-
Y.9	+	+	s	w	w	s	+	+	w	w	w	+	w	W	-
Y.10	+	s	+	w	w	s	s	+	w	w	w	w	+	+	-
Y.13	+	+	s	w	w	s	+	+	w	w	w	+	w	W	-
Y.14	+	w	-	+	w	w	-	+	-	-	-	w	w	W	-
Y.15	+	w	-	+	w	+	-	s	-	-	-	w	+	+	-
Y.16	+	-	-	s	w	+	+	w	+	-	-	+	+	+	-

Symbols: (+) positive; (-) Negative; (s) Strong positive; (w) Weak positive; Data obtained from three independent experiments

Table 3. Fermentation abilities of selected yeasts isolates

Isolate no.	Glucose	Galactose	D-xylose	Maltose	Lactose	Sucrose	Raffinose
Y.2	+	+	-	w	-	+	+
Y.5	+	-	-	-	-	-	-
Y.6	+	-	-	-	-	-	-
Y.7	W	w	-	-	-	-	-
Y.8	+	w	-	-	-	-	-
Y.9	+	w	-	-	-	-	-
Y.10	+	w	-	-	-	-	-
Y.13	+	w	-	-	-	-	-
Y.14	-	-	-	-	-	-	-
Y.15	-	-	-	-	-	-	-
Y.16	-	-	-	-	-	-	-

Symbols: (+) positive; (-) Negative; (s) Strong positive; (w) Weak positive. Data obtained from three independent experiments

Table 4. Additional biochemical tests using isolated yeasts

Strain no.	L-lysine	Nitrite	Nitrate	Growth at 30℃	Growth at 37℃	Starch formation	Glucose 50%	Glucose 60%	NaCl 10%+ glucose 5%	Acid production	Hydrolysis of urea	Acetic acid 1%	Starch hydrolyses
Y.2	-	-	-	+	+	-	+	w	w	+	-	-	-
Y.5	-	+	+	+	+	-	-	-	-	+	s	-	W
Y.6	s	-	-	+	s	-	+	-	w	s	w	-	-
Y.7	+	-	-	+	-	-	s	s	w	-	w	-	-
Y.8	+	-	-	+	-	-	s	s	w	w	+	-	-
Y.9	+	-	-	+	-	-	s	s	w	w	+	-	-
Y.10	w	-	-	+	-	-	s	s	w	w	s	-	-
Y.13	+	-	-	+	-	-	s	s	w	w	+	-	-
Y.14	-	-	-	+	+	-	w	-	-	-	s	-	-
Y.15	w	-	-	+	+	-	w	-	-	-	s	-	-
Y.16	s	-	-	-	-	+	+	-	+	-	s	-	-

Symbols: (+) positive; (-) Negative; (s) Strong positive; (w) Weak positive. Data obtained from three independent experiments

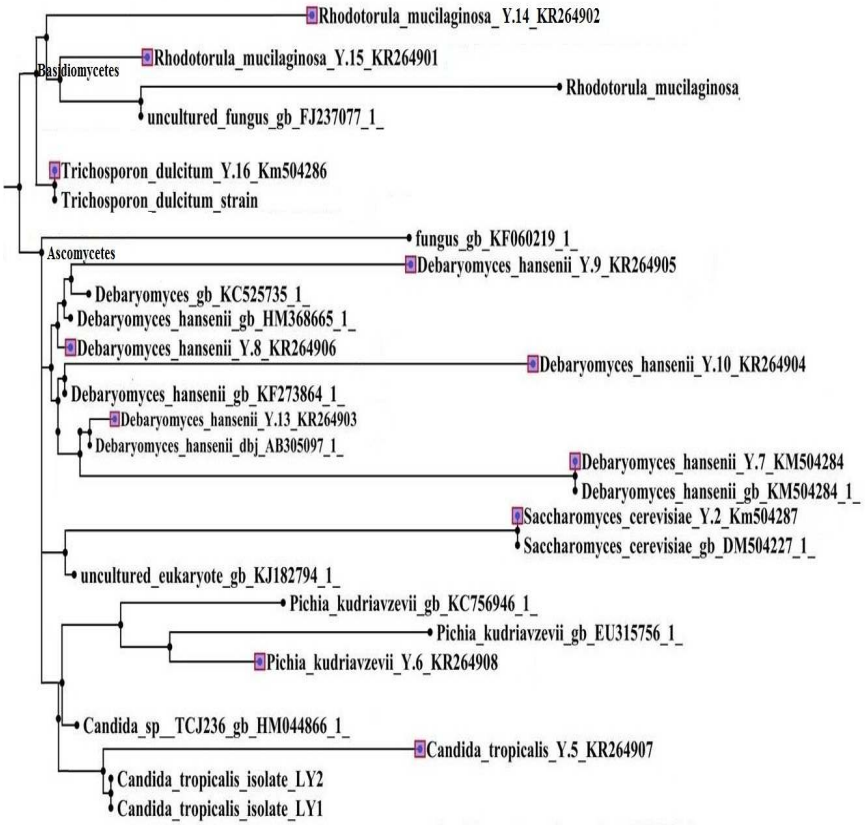

Fig. 1. Phylogenetic tree relationships of rRNA sequences genes of identified yeasts

Table 5. Identification of yeast strains based on molecular bases and their accession numbers

Strain no.	Source	Accession number	Strain name
Y.2	Sugarcane juice	KM504287	*Saccharomyces cerevisiae*
Y.5	Egyptian Sobia drink	KR264907	*Candida tropicalis*
Y.6	Yogurt	KR264908	*Pichia kudriavzevii*
Y.7	Orange	KM504284	*Debaryomyces hansenii*
Y.8	Mandarin	KR264906	*Debaryomyces hansenii*
Y.9	Tomato	KR264905	*Debaryomyces hansenii*
Y.10	Squash	KR264904	*Debaryomyces hansenii*
Y.13	Mango juice	KR264903	*Debaryomyces hansenii*
Y.14	Mango juice	KR264902	*Rhodotorula mucilaginosa*
Y.15	Mango juice	KR264901	*Rhodotorula mucilaginosa*
Y.16	Buttermilk	KM504286	*Trichosporon dulcitum*

The complex identification yeasts by a conventional methodology of biochemical that requires 60~90 tests for correct species identification [22]. Nonetheless, these methodology are necessary for detect any special physiological character. Furthermore, due to high accuracy of identification by molecular techniques, it was used in parallel with biochemical tests for the identification of yeast species. The obtained results from morphological characteristics of the isolated colonies coupled with the biochemical tests were highly significant in yeast identification and correlated with molecular technique identification.

D. hansenii showed the most frequented strain where five isolates of *D. hansenii* isolated from different sources (fruit samples and mango juice). It has an ability to assimilate all tested sources of carbon except methanol, in addition to, ferment glucose and galactose. Galactose is a 'non-conventional' nutrient for yeasts, which however can be used as a sole carbon source when glucose is absent from the medium. Thus, the ability of the yeast cells to assimilate galactose indicated the expression of the GAL genes [23]. As a consequent of that, *D. hansenii* may has GAL genes. It has caused spoilage of fruit, juice, marzipan, and canned figs [24,25]. It has been isolated from a variety of fruit [24], soft drinks [26], raw tomatoes [27], sugarcane [28] and been reported in high numbers in yoghurt [29]. It proved to be genetically and biochemically interesting yeasts with considerable biotechnological promise [30].

R. mucilaginosa is the second most frequented strain. The results in consistent with Haridy [31], where *Rhodotorula* spp. are common strains isolated from dairy products. The presence of *R. mucilaginosa* in the mango juice signified cross-contamination during juice preparation because *Rhodotorula* species have been reported as a saprophyte skin, vaginal and respiratory specimens [32]. Contamination may also occur through contaminated containers because all members of the *Rhodotorula* genus, which has affinity to synthetic materials in general [33]. The incidence of *R. mucilaginosa* in the mango juice is very dangerous because it has been reported to cause Onychomycosis, which is a dermatological problem in immunocompetent patient [34].

P. kudriavzevii was associated only with yogurt during our study. It initially classified as a strain of *Issatchenkia orientalis*, but Kreger-van Rij [35] noted that this strain (CBS 5459) differed from the others in being sorbose-positive and citrate-negative. An isolated strain has an ability to assimilate glucose, galactose, maltose, sucrose, ethanol and glycerol, in addition to, ferment only glucose. *P. kudriavzevii* gained much attention recently in the biotechnology. Recombinant *P. kudriavzevii* VTT C-79090T had been able to produce up to 171 g L^{-1} of D-xylonate from 171 g L^{-1} D-xylose at low pH [36]. Yuangsaard et al. [37] reported that newly isolated thermotolerant *Pichia kudriavzevii* had been produced 7.86% (w/v) ethanol from cassava starch hydrolysate, which adjusted to 18% glucose at 40℃ within 24 h. Moreover, the ability of *P. kudriavzevii* to utilize glycerol makes it an excellent source for biodiesel production. Recently, Thiru et al. [38] have carried out a detailed investigation on the production of biodiesel from crude glycerol using *Cryptococcus curvatus*. *S. cerevisiae* was isolated from sugarcane juice, *C. tropicalis* from Egyptian sobia drink, and *T. dulcitum* from buttermilk. Mendoza et al. [39] stated that *Rhodotorula*, *Pichia*, *Candida,* and *Saccharomyces* were

frequently isolated from pasteurized fruit juices while; *Candida* and *Saccharomyces* spp. have often been reported as spoilage-causing organisms in citrus juices [40,41].

5. CONCLUSION

This study reported the occurrence of yeast species associated with dairy products, fruits, and juices collected from Assiut City, Egypt. Eleven yeast isolates belonged to six genera were successfully identified. Four species belonged to ascomycetes: *D. hansenii* (five isolates), *S. cerevisiae* (one isolate), *C. tropicalis* (one isolate), and *P. kudriavzevii* (one isolate). In addition, two yeasts species belonged to basidiomyces: *R. mucilaginosa* (two isolates), and *T. dulcitum* (one isolate). The biochemical identification was highly consistent with molecular identification techniques using amplified fragments of ITS and D1/D2 domains of large subunit. Dairy products, fruits, juices were commonly associated by different yeasts and it can be used for isolation of different genera and species.

ACKNOWLEDGEMENTS

We greatly thank Dr. Tsutomu Kodaki, Institute of Advanced Energy, Kyoto University, Japan for facilitate uses of Applied Biosystems 3031 genetic analyzer and ABI Prism BigDye Terminator v3.1 Cycle Sequencing kit.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Bekatorou A, Psarianos C, Koutinas AA. Production of food grade yeasts. Food Technology and Biotechnology. 2006;44: 407-415.
2. Starmer WT, Lachance MA. Yeast ecology. In: The Yeasts A Taxonomic Study. 5th ed. Kurtzman CP. Fell JW. Boekhout T. Elsevier: Amsterdam. 2011; 65-86.
3. Spencer DM, Spencer JFT, De Figueroa L, Heluane H. Yeasts associated with rotting citrus fruit in Tucuman, Argentina. Mycological Research. 1992;96:891-892.
4. Vacek DC, Starmer WT, Heed WB. Relevance of the ecology of citrus yeasts to diet of *Drosophila*. Microbial Ecology. 1979;5:43-49.
5. Suresh ER, Onkarayya H, Ethiraj SD. A note on the yeast flora associated with fermentation of mango. Journal of Applied Bacteriology. 1982;52:1-4.
6. Jager ES, Wehner FC, Korsten L. Microbial ecology of the mango phylloplane. Microbial Ecology. 2001;42: 201-207.
7. Lachance MA, Starmer WT, Rosa CA, Bowles JM, Barker JSF, Janzen DH. Biogeography of the yeasts of ephemeral flowers and their insects. FEMS Yeast Research. 2001;1:1-8.
8. Berry JM. Yeast problems in the food and beverage industry. In: Food Mycology ed. (Rhodes, ME) GK Hall Co., Boston, MA. 1979;82-90.
9. Deak T. Hygienic and technological consideration of yeasts in the soft drink industry. Acta Alimentaria. 1980;9:90-91.
10. Wood BJ. Microbiology of fermented foods, 2nd ed., vol 1, London: Blackie Academic Professional. 1998;96.
11. El-Sharoud WM, Belloch C, Peris D, Querol A. Molecular identification of yeasts associated with traditional Egyptian dairy products. Journal of Food Science. 2009;74(7):341-346.
12. Soliman NSM, Aly SA. Occurrence and identification of yeast species isolated from Egyptian Karish cheese. Journal of Yeast and Fungal Research. 2011;2(4):59-64.
13. Lachance MA, Starmer WT. Ecology and yeasts. In: Kurtzman CP, Fell JW, editors. The yeasts a taxonomy study, 4th ed. Elsevier: Science BV. Amsterdam: Netherlands. 1998;21-30.
14. Lodder J, Kreger-van Rij NJW. The yeasts a taxonomic study. North-Holland Amsterdam; 1952.
15. Kurtzman CP, Fell JW, Boekhout T, Robert V. Methods for isolation phenotypic characterization and maintenance of yeasts. In: Kurtzman CP, Fell JW, Boekhout T. editors. The Yeasts a Taxonomic Study 5th ed. Elsevier; 2011.
16. Van der Walt JP, Yarrow D. Methods for isolation maintenance classification and identification of yeasts. In: Kreger-van Rij JW, editor. The yeasts: A taxonomic study. 3rd ed. Elsevier: Amsterdam; 1984.
17. Nisiotou AA, Spiropoulos AE, Nychas GJE. Yeast community structures and dynamics in healthy and botrytis-affected grape must

fermentations. Applied and Environmental Microbiology. 2007;9:6705-6713.
18. Kurtzman CP, Fell JW. Summary of species characteristics In: The yeasts a taxonomic study 4th ed. Kurtzman CP, Fell JW, editors. Amsterdam; Elsevier; 1998.
19. Hanna M, Xiao W. Isolation of nucleic acids in: Xiao W, (Ed) Yeast Protocols 2nd ed. Vol. 313 Humana Press New Jersey. 2000;15-20.
20. Khattab SMR, Kodaki T. A novel production method for high-fructose glucose syrup from sucrose-containing biomass by a newly isolated strain of osmotolerant *Meyerozyma guilliermondii*. Journal of Microbiology and Biotechnology; 2015. (In press).
DOI: 10.4014/jmb.1510.10091
21. Banat IM, Nigam P, Singh D, Marchant R, McHale AP. Review: Ethanol production at elevated temperatures and alcohol concentrations: Part I – Yeasts in general World Journal of Microbiology and Biotechnology. 1998;14:809-821.
22. Deák T. Methods for the rapid detection and identification of yeasts in foods. Trends Food Science Technology Journal. 1995;6:287-292.
23. Yun CW, Bauler M, Moore RE, Klebba PE, Philpott CC. The role of the FRE family of plasma membrane reductases in the uptake of siderophore iron in *Saccharomyces cerevisiae*. Journal of Biological Chemistry. 2001;276:10218-10223
24. Deak T, Beuchat LR. Handbook of food spoilage yeasts. Boca Raton Fla: CRC Press; 1996.
25. Pitt JI, Hocking AD. Fungi and Food Spoilage. Aspen Publishers. Inc Gaithersburg MD: USA; 1999.
26. Ancasi EG, Carrillo L, Benitez Ahrendts MR. Moulds and yeasts in bottled water and soft drinks. Revista Argentina De Microbiologia. 2006;38:93-96.
27. Wade WN, Vasdinnyei R, Deak T, Beuchat LR. Proteolytic yeasts isolated from raw ripe tomatoes and metabolic association of *Geotrichum candidum* with *Salmonella*. International Journal of Food Microbiology. 2003;86:101-111.
28. Azeredo LAI, de Gomes EAT, Mendonca-Hagler LC, Hagler AN. Yeast communities associated with sugarcane in Campos, Rio de Janeiro, Brazil. International Microbiology. 1998;1:205-208.
29. Yamani MI, Abu-Jaber MM. Yeast flora of labaneh produced by in-bag straining of cow milk set yogurt. Journal of Dairy Science. 1994;77:3558-3564.
30. Baronian KHR. The use of yeasts and moulds as sensing elements in biosensors. Biosensors and Bioelectronics. 2004;19: 953-962.
31. Haridy MSA. Yeast flora of raw-milk in El-Minia-City, Egypt. Cryptogamie Mycologie. 1992;13:321-326.
32. Gomez-Lopez A, Mellado E, Rodriguez-Tudela JL, Cuenca-Estrella M. Susceptibility profile of 29 clinical isolates of *Rhodotorula* spp. Journal of Antimicrobial agent and Chemotherapy. 2005;55(3):312-316.
33. Goyal R, Das S, Arora A, Aggarwal A. *Rhodotorula mucilaginosa* as a cause of persistent femoral nonunion. Journal of Postgraduate Medicine. 2008;54:25-27.
34. Marcel ML, Luana PB, Marcos DR, Alane BV, Sonia R. Identification antifungal susceptibility and scanning electron microscopy of a keratinolytic strain of *Rhodotorula mucilaginosa*: A primary causative agent of onychomycosis. FEMS Immunology and Medical Microbiology. 2009;55(3):396-403.
35. Kreger-van Rij NJW. Debaryomyces Lodder and Kreger-van Rij nom conserve. In: Lodder J, editor. The yeasts: A taxonomic study. 2nd ed. North-Holland Amsterdam; 1970.
36. Toivari M, Vehkomäki ML, Nygård Y, Penttilä M, Ruohonen L, Wiebe MG. Low pH D-xylonate production with *Pichia kudriavzevii*. Bioresource Technology. 2013;133:555–562.
37. Yuangsaard N, Yongmanitchai W, Yamada M, Limtong S. Selection and characterization of a newly isolated thermotolerant *Pichia kudriavzevii* strain for ethanol production at high temperature from cassava starch hydrolysate. Antonie van Leeuwenhoek. 2013;103:577-588.
38. Thiru M, Sankh S, Rangaswamy V. Process for biodiesel production from *Cryptococcus curvatus*. Bioresource Technology. 2011;102:10436–10440.
39. Mendoza S, Montemayor L, Boscan LA, Barreiro JA. Microflora in pasteurized fruit juices in Venezuela. Archivos Latinoamericanos de Nutrición. 1982; 32(3):617-629.

40. Parish ME, Higgins DP. Yeasts and molds isolated from spoiling citrus products. Journal of Food Protection. 1989;52:261-263.

41. Teller KH, Parish MH. Microbially produced offflavors in orange juice. Proceeding Florida State Horticultural Society. 1992;105:144-146.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/13348