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Thirteen New Yeast Strains Isolated from Cancer Patients in Basrah-Iraq by ITS rDNA Sequencing

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Abstract

Yeast isolates were grown on CHR OMagar Candida medium to detect different types of the yeast species, and then the unidentified isolates were diagnosed by using molecular analysis of ITS region. From a total of 54 yeast isolates, 37 isolates appeared as different species of the genus Candida, the rest of species belong to Hanseniaspora, Kazachstania, Kluyveromyces, Magnusiomyces, Pichia and Saccharomyces. 13yeasts species in this study reported as new strains in Gen Bank, also species belong to C. pronicula, H. uvarum, K. exigua, K. marxianus, M. capitatus, Magnusiomyces sp., P. kudriavzevii, P. manshurica and S. bayanus x S. cerevisiae isolated for the first time in Iraq. We concluded that there are new emergent species of the yeasts inhabit the oral cavity of cancer patients undergoing chemotherapy, and the results showed that the molecular method provide a good approach for the identification.

Key words: *yeasts, cancer patient, molecular identification.*

Introduction

Certain fungi, notably some of Candida species, are inhabited the oral cavity as a commensals, but the alteration of mouth environment with certain virulence determinants lead to overgrowth of fungal species causing oral infections. The incidence of oral candidiasis has been reported to be ranging from 7-52% among patients with different types of malignancies (1,2).

Cancer patients undergoing chemotherapy treatment are prone to higher risk for fungal infections because of the host immunosuppression as the action of these drugs, the identification of the causative agent requires rapid and accurate methods to identify the pathogen for guiding appropriate therapy. Molecular identification of fungal pathogens often involves polymerase chain reaction (PCR) for ITS1-5.8S-ITS2 rDNA gene (3). The non-coding complex ITS region is evolved more rapidly and variable among different species within a genus, therefore it is appropriate for taxonomy and identification than the coding and conserved 5.8S rDNA gene. ITS region has typically been most useful for molecular systematics at the species and strains level. (4,5).

The present study was conducted to detection and discrimination of the yeasts at the strains level, isolated from oral cavity of cancer patients, by using molecular genetic analysis of ITS region.

Materials and Method

500 samples were obtained from the lining of the oral cavity of patients (195 males and 305 females) with different types of cancer, were submitted to chemotherapy at Al-Sader Teaching Hospital-Basrah, Iraq, in the period between December 2014 to February 2015.

The yeast isolates were identified by CHRO Magar Candida medium to distinguish between the different species by type and color of the colonies (6).

From the total isolates which identified by conventional methods, there were 54 isolates their identification still uncertain at the species level, so we used the molecular methods for the purpose of accurate identification .

According to (7), the DNA extraction and PCR amplification has been accomplished, to amplify the internal transcribed spacer regions (ITS) of ribosomal

DNA with a 19 base forward primer, ITS1 (5'-TCC GTA GGT GAA CCT GCG G-3') and a 20 base reverse primer, ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3'). The result of PCR technique yielded distinctive products size of approximately 400-850 bp.

The purification and protocol of PCR products for 54 samples were carried at MACROGEN Company http://dna.macrogen.com. All the alignments were observed by Clustal Omega www.clustal.org./omega/ and identified using Blast program.

Results

The amplified ITS regions were appeared in all 54 yeast isolates accurately. The identification of gene sequences showed 15 yeast species belong to 7 genera (Table 1).

Table 1: Genus and species of the yeasts identified by ITS1- 5.8S-ITS2 rDNA gene sequencing

No.	Genus	Species	Number of Isolates
1	Candida	Albicans	10
		Dubliniensis	11
		Glabrata	7
		Parapsilosis	6
		Prunicola	1
		Tropicalis	2
2	Hanseniaspora	Uvarum	1
3	Kazachstania	Exigua	1
4	Kluyveromyces	Marxianus	2
5	Magnusiomyces	sp.	1
		Capitatus	2
6	Pichia	Kudriavzevii	4
		Manshurica	1
7	Saccharomyces	bayanus X S. cerevisiae	1
		Cerevisiae	4

Out of 54 different isolates identified by PCR sequencing there were 13 isolates reported and published as new strains in Gen Bank (NCBI) (Table 2).

Table 2: Comparison between conventional and molecular identification for the yeast isolates

No.	No. of isolate	Species		Similarity %	Name of new strain
		Conventional identification	Molecular identification		
1	2	Candida dubliniensis	Candida albicans	100	-
2	12	Candida glabrata	Candida albicans	100	-
3	16	Candida albicans	Candida albicans	100	-
4	27	Candida albicans	Candida albicans	99	IQNasir23 *
5	31	Candida parapsilosis	Candida albicans	100	-
6	39	Candida glabrata	Candida albicans	99	IQMunaff29 *
7	47	Candida tropicalis	Candida albicans	100	-

Cont... **Table 2: Comparison between conventional and molecular identification for the yeast isolates**

8	48	Candida krusei	Candida albicans	100	-
9	51	Candida albicans	Candida albicans	99	IQTech.Ins,32 *
10	53	Candida tropicalis	Candida albicans	100	-
11	8	Candida dubliniensis	Candida dubliniensis	100	-
12	10	Candida tropicalis	Candida dubliniensis	100	-
13	13	Candida tropicalis	Candida dubliniensis	100	-
14	21	Candida glabrata	Candida dubliniensis	100	-
15	22	Candida dubliniensis	Candida dubliniensis	100	-
16	23	Candida dubliniensis	Candida dubliniensis	100	-
17	24	Candida dubliniensis	Candida dubliniensis	100	-
18	29	Candida tropicalis	Candida dubliniensis	100	-
19	34	Candida tropicalis	Candida dubliniensis	100	-
20	46	Candida dubliniensis	Candida dubliniensis	100	-
21	52	Candida albicans	Candida dubliniensis	100	-
22	1	Candida glabrata	Candida glabrata	100	-
23	26	Candida krusei	Candida glabrata	100	-
24	15	Candida glabrata	Candida glabrata	100	-
25	33	Candida parapsilosis	Candida glabrata	100	-
26	36	Candida parapsilosis	Candida glabrata	99	IQMuhanad26 *
27	37	Candida glabrata	Candida glabrata	99	IQAbdulla27 *
28	38	Candida tropicalis	Candida glabrata	99	IQBasrah28 *
29	3	Candida krusei	Candida parapsilosis	100	-
30	4	Candida krusei	Candida parapsilosis	100	-
31	6	Candida parapsilosis	Candida parapsilosis	100	-
32	9	Candida glabrata	Candida parapsilosis	100	-
33	19	Candida krusei	Candida parapsilosis	100	-
34	44	Candida parapsilosis	Candida parapsilosis	99	IQMustafa31 *
35	30	Candida glabrata	Candida prunicola	98	IQLamyaa24 *
36	11	Candida tropicalis	Candida tropicalis	100	-
37	41	Candida glabrata	Candida tropicalis	99	IQMunaff30 *
38	35	Candida glabrata	Hanseniaspora uvarum	100	-
39	5	Candida parapsilosis	Kazachstania exigua	99	IQBashar21 *
40	18	Candida parapsilosis	Kluyveromyces marxianus	100	-
41	50	Candida parapsilosis	Kluyveromyces marxianus	100	-
42	17	Candida krusei	Magnusiomyces capitatus	100	-
43	28	Candida albicans	Magnusiomyces capitatus	100	-
44	45	Candida glabrata	Magnusiomyces sp.	100	-
45	14	Candida krusei	Pichia kudriavzevii	100	-
46	20	Candida krusei	Pichia kudriavzevii	100	-
47	43	Candida glabrata	Pichia kudriavzevii	100	-
48	49	Candida krusei	Pichia kudriavzevii	100	-
49	42	Candida krusei	Pichia manshurica	100	-
50	7	Candida glabrata	Saccharomyces cerevisiae	92	Untreated
51	25	Candida albicans	Saccharomyces cerevisiae	99	IQMustafa22 *
52	32	Candida glabrata	Saccharomyces cerevisiae	99	IQAlsaadoon25 *
53	40	Candida krusei	Saccharomyces cerevisiae	93	Untreated
54	54	Candida glabrata	Saccharomyces bayanus X S. cerevisiae	99	IQBashar33 *

* New strains

Discussion

The accurate and timely species identification is important for clinical management of patients because the yeast species have different antifungal susceptibilities, that is why the choice of the ITS1-5.8S-ITS2 rDNA gene sequencing for discrimination of the species is important (8,9,10).

The universal primers ITS1 and ITS4 were used for amplifying ITS1 and ITS2 regions in yeast species, these primers showed high efficiency appeared in the identification of 13 new yeast strains, some of them have been isolated and reported for the first time from the oral cavity of cancer patients such as C. prunicola, K. exigua, K. marxianus and P. manshurica that could be due to the changes of the mouth environment and/or influence of chemotherapy drugs which acts as immunosuppressive then alteration the lining of oral cavity that upset the healthy balance of the oral microorganisms allowing to fungal infection (11,12).

Also, to our knowledge, different uncommon yeasts were isolated as new record species from oral cavity for the first time in Iraq: C. pronicula, H. uvarum, K. exigua, K. marxianus , M. capitatus, Magnusiomyces sp., P. kudriavzevii, P. manshurica and S. bayanus x S. cerevisiae, this appearance could be due to suppression of the host immune system as a result of the cancer disease, use of chemotherapy drugs which suppressed oral/mucosal immunity, salivary gland dysfunction and alteration in oral flora leading to change the quantity and quality of saliva that encourage infection by opportunistic fungi as well as lack of physical activity and a high carbohydrate diet (2,13,14).

This identification were agreed with previous studies of (1,9,15,16), which isolated different species of Candida based on ITS region sequencing. The present study showed similar results to those observed from (17), who reported six of Candida species from cancer patients.

H. uvarum is considered as opportunistic yeast and its occurrence in clinical isolates is unusual, but in this study, the appearance of this species in the oral cavity is considered the first recording as a result of malignancies and chemotherapy impact . It was first isolated in Spain by (18) from the oral cavity of a 70 year old women with lesions produced by the dentures, and they suggested that the source of infection was associated to handling and consumption of raw fish (19).

Although K. marxianus is less frequently in clinical samples but its prevalence has increased over the past decade. The anamorph form of this species (C. kefyr) is an emerging pathogen with hematological malignancies (11), while in current study the result was not consistent with that previous study because two strains of K. marxianus were isolated from the oral cavity of females with breast cancer.

C. prunicola, P. manshurica and S. cerevisiae were isolated from clinical samples by (20), but to our knowledge, the present study considered the first work to isolate them from the oral cavity of cancer patients and identification them through molecular methods.

The study of (21) described a case with M. capitatus infection in non-neutropenic patient. M. capitatus is an opportunistic pathogen often observed in immunocompromised patients. The effect of cytotoxic chemotherapy, which considered immunosuppression agents, leads to development of infections in cancer patients.

In spite of the teleomorph species K. exigua is unlikely to be human pathogen because it did not grow at 37℃ but in this study it was isolated for the first time from oral cavity. The fungal sexually reproduction in human body have a higher enzymatic activity and thus greater the virulence of the host tissues, that explains the increased appearance of teleomorph conditions in clinical samples (22).

P. kudriavzevii, which a teleomorph state of C. krusei, is abundant in the environment and mainly associated with food spoilage. Many studies have shown that P. kudriavzevii is the 5th most common cause of candidemia in immunocompromised patients (23). To our knowledge, this study consider the first work that isolated the teleomorph condition of C. krusei from oral cavity of cancer patients.

Conclusions

Many yeasts species showed a high similar phenotypic features, so molecular methods by using amplification of ITS1-5.8S-ITS2 rDNA gene provide a good alternative approach for the identification of pathogenic yeasts.

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References

1- Elena R , Simona ES , Diana P , Ionela S , Cojocaru M , Tatiana V. Identification of species of genus Candida by analysis of 5.8S rRNA gene. Romanian Biotechnological Letters. (2015); 20(4): 10585-10591.

2- Jayachandran AL , Katragadda R , Thyagarajan R , Vajravelu L , Manikesi S , Kaliappan S , Jayachandran B. Oral candidiasis among cancer patients attending a tertiary care hospital in Chennai, South India: An evaluation of clinic mycological association and antifungal susceptibility pattern. Canadian Journal of Infectious Diseases and Medical Microbiology. (2016); 1-6.

3- Imran ZK , Ali EK. Molecular identification of Candida glabrata and C. parapsilosis based on sequencing analysis of rDNA. Valley International Journal. (2015); 2(12): 1490-1497.

4- Ali HH , Al-Obaidi RN , Fattah CH . Molecular identification of Candida species isolated from ears of dogs infected with otitis externa by detecting Internal Transcript Spacer (ITS1 and ITS2) in Sulaimania, Iraq. Advances in Animal and Veterinary Sciences Journal. (2015); 3(9): 491-499.

5 - Teoh F , Pavelka N. How chemotherapy increases the risk of systemic candidiasis in cancer patients: Current paradigm and future directions. Pathogens. (2016); 5(6): 1-16.

6- Aldossary MA , Almansour NA , Abdulraheem BS . Isolation and identification of Candida species from the oral cavity of cancer patients undergoing chemotherapy in Basrah, Iraq. Journal of Biology Agriculture and Healthcare. (2016); 6(18): 22-30.

7- Mirhendi H , Makimura K , Khoramizadeh M , Yamaguchi H. A one-enzyme PCR-RFLP assay for identification of six medically important Candida species. Japanese Journal of Medical Mycology. (2006); 47(3): 225-229.

8- Daef E , Moharram A , Saif-Eldin S , Elsherbiny N. Evaluation of chromogenic media and seminested PCR in the identification of Candida species. Brazilian Journal of Microbiology. (2014); 45(1): 1-9.

9- Karimi L , Mirhendi H , Khodadadi H , Mohammadi R. Molecular identification of uncommon clinical yeast species in Iran. Current Medical Mycology. (2015); 1(2): 1-6.

10- Hou X , Xiao M , Chen SCA , Wang H , Zhang L , Fan X , Xu Z , Cheng JW , Kong F , Zhao YP, Xu YC. Sequencer-based capillary gel electrophoresis (SCGE) targeting the RDNA Internal Transcribed Spacer (ITS) regions for accurate identification of clinically important yeast species. PLOS ONE. (2016); 11(4): 1-16.

11- Dufresne SF , Marr KA , Sydnor E , Staab J F , Karp JE , Lu K , Zhang SX , Lavallee C , Perl TM , Neofytos D. Epidemiology of Candida kefyr in patients with hematologic malignancies. Journal of Clinical Microbiology. (2014); 52(6): 1830-1837.

12- Jain M , Shah R , Chandolia B , Mathur A , Chauhan Y , Chawda J , Mosby S , Bhagalia S. The oral carriage of Candida in oral cancer patients of Indian origin undergoing radiotherapy and/or chemotherapy. Journal of Clinical and Diagnostic Research. (2016); 10(2):17-20.

13- Alt-Epping B , Nejad RK , Jung K , Grob U, Nauck F. Symptoms of the oral cavity and their association with local microbiological and clinical findings-a prospective survey in palliative care. Supportive Care in Cancer. (2012); 20(3): 531-537.

14- Pattar V , Nalaband Z , Bagewadi A. Oral presentation of chronic hyperplastic candidiasis in patient under Imatinib Mesylate: A rare case. International Journal of Scientific Study. (2016); 2(11): 17-19.

15- Barbedo LS , Figuerpiedo-Carvalho MHG , Muniz MM , Zancope-Oliveira RM. The identification and differentiation of Candida parapsilosis complex species by polymerase chain reaction-restriction

fragment length polymorphism of internal transcribed spacer region of the DNA. Memorias do Instituto Oswaldo Cruz. Rio de Janeiro. (2016); 111(4): 267-270.

16- Benedetti VP , Savi DC , Aluizio R , Adamoski D, Kava-Cordeiro V , Galli-Terasawa LV , Glienke C. Analysis of the genetic diversity of Candida isolates obtained from diabetic patients and kidney transplant recipients. Memorias do Instituto Oswaldo Cruz. Rio de Janeiro. (2016); 111(7): 417-422.

17- Sousa LVNF , Santos VL , Monteiro AS , Souza MVD , Marques SG , Faria ES , Assuncao EAO , Santos SG , Zonis JM , Alvarenga DG , Holanda RA , Sousa JG , Santos KV , Stoianoff MAR. Isolation and identification of Candida species in patients with orogastric cancer: susceptibility to antifungal drugs, attributes of virulence in vitro and immune response phenotype. BMC Infectious Diseases. (2016); 16(86): 1-12.

18- Garcia-Martos P, Hernandez-Molina JM , Galan F , Ruiz-Henestrosa JR , Garcia-Agudo R , Palomo MJ , Mira J. Isolation of Hanseniaspora uvarum (Kloeckera apiculata) in humans. Mycopathologia. (1999); 144: 73-75.

19- Albertin W , Setati ME , Miot-Sertier C , Mostert TT , Colonna-Ceccaldi B , Coulon J , Girard P , Moine V , Pillet M , Salin F , Bely M , Divol B , Masneuf-Pomarede I. Hanseniaspora uvarum from winemaking environments show spatial and temporal genetic clustering. Frontiers in Microbiology Journal. (2016); 6: 1-16.

20- Cassagne C , Normand A , Bonzon L , Ollivier C , Gautier M , Jeddi F , Ranque S , Piarroux R. Routine identification and mixed species detection in 6192 clinical yeast isolates. International Society for Human and Animal Mycology Journal (ISHAM). (2016); 54: 256-265.

21- Brunetti G , Visconti V , Ghezzi MC , Mantovani S , Ferretti G , Raponi G. Management and treatment of Magnusiomyces capitatus (Geotrichum capitatum) pleural infection in a non-neutropenic patient with posaconazole. A new therapeutic opportunity. New Microbiologica. (2016); 39(4): 307-309.

22- Goralska K , Klimczak A , Rachubinski P , Jaglowska A , Kwapiszewska A. Consumption of sweetened beverages as a risk factor of colonization of oral cavity by fungi-eating habits of university students. Annals of Parasitology. (2015); 6(3): 175-182.

23- Mbuk EU , Kwaga JKP , Bale JOO , Umoh JU. Molecular identification of yeasts associated with raw cow milk from peri-urban farms in Kaduna State, Nigeria. Journal of Yeast and Fungal Research. (2016); 7(5): 39-46.