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Original Article

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Molecular Diagnosis of Pathogenic Fungi Isolated from the Skin and Mouth

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Abstract:

Background: This study was conducted in the Postgraduate Laboratory at the College of Sciences at the University of Kerbala, from patients with dermatophytes ,where 115 sample were collected for the period from December 2022 until May 2023 where 100 sample were collected ,isolated from patients with dermatophytes which were taken from skin ,nails and hair in Imam Al-Hassan -Almujtaba Hospital ,peace be upon him , in Karbala Governoate, and 15 samples were taken from the mouth at the childrens teaching hospital. the samples were directly direct microscopic examination and by observing the external appearance of the colonies after culturing them on medium of Saparoid dextrose agar ,the culture result showed two types of dermatophytes and atype of Candida yeast (*Trichophyton indotinea*, *Trichophyton qunickeueam* and *Candida albicans*.)

Objectives:Diagnosis of pathogenic fungi isolated from the skin and mouth molecularly because it is more accurate and specialized than the phenotypic diagnostic and evolutionary tree drawing to find the relationship between locally isolated fungi and global isolates. **Materal and Methods** : sampla was culture on saporoud dextrose agar,after being diagnosed phenotypic diagnosed molecular examination the DNA was extracted from the mentioned pathogenic fungi using aKorean extraction kit(Favrogene extraction kit for fungi) and then the migration was carried out by electrophoresis,then the DNA molecule is amplified using the polymerase chain reaction(pcr) .

The ITS region was adopted as the best Barcording region in the diagnosis of pathogenic fungi. The Primer pairs ITS4&ITS5 were adopted which is ageneral pair targeting the location of the gene sequence.

Result: the result of the polymerization showed unique bands that ranged between 510-850 base pair.

Conclusion:the result of the current study showed that the genetic tree of the Dermatophytosis and Candida yeast taken from the mouth ,and after send the results to the Gen Bank to compare them with recorded fungi at this site and using a program MEGA(multiple alignument analysis tool6.0).the result showed a clear convergence between the local fungi isolates compared to the rest of the isolates present in the tree.

Key wards : extraction, electrophoresis, Barcording region

Introduction

Dermatophytes represent the main cause of cutaneous diseases. Dermatophytes attack keratinized tissues, such as hair, nail , and stratum corneum, through its ability to produce keratinolytic enzymes, which leads to dermatophytosis. line of defense for the body health problemes in developing countries ,as fungal infection constitute a high percentage of the causes of skin infection , affecting all age groups and different parts of the body by three species of filamentous fungi belong to the Euascomycetes class ;Trichophyton ,Microsporum and Epidermopyton) 1.(

Transmission of dermatophytes may occur by direct contact with animals or humans or indirectly by contact with contaminated fomites(2). The factors that contribute to a high incidence of superficial mycoses, especially in tropical and subtropical regions are heat and humid (3)(4).

the phenotypic diagnosis is of great importance, but it requires a high level of experience and a great deal of effort and time .now , the diagnosis of fungi using the polymerase chain reaction technology is characterized by high accuracy and avoid the problems of diagnosis by traditional method (5) Accurate and rapid diagnosis using molecular methods helps in identifying pathogenic fungi and thus increases the chance of recovery by using appropriate antifungal therapy) 6 .(

Materials and methods

A total of 115 specimens were collected from patients with dermatophytosis in Imam Hassan Al-Mujtaba Hospital in Karbala , the specimens collected from skin , hair, nail and from the mouth of theChildrens teaching hospital ,dermatophytes isolated can be diagnosed by direct microscopic examination with 15%-20% KOH and using physiological test such as vitamin requirement test, growth on rice,hair perforation test and producing pigments in other culture media. the ITS region was adopted as the best molecular marker in the diagnosis of fungi.

To apply this technique, DNA extraction was required from dermatophytes and Candida yeast taken from the mouth .the results of DNA extraction showed varying concentrations between isolates due to the different species and genas of pathogenic fungi. We extracted DNA from fungi using the Korean -origin Favrogene extraction kit .

Then we carry out the electrophoresis process , which is defined as the movement of ions and charged giant molecules such as DNA,RNA and protein through a specific medium such as agarose gel or polyacrylamide gel ,which occurs when an electric current is applied.

DNA and RNA molecules carry a negative charge because of their phosphate -containing back bone which will migrate and be attracted towards the positive pole ,(the anods).as the proteins ,they carry different charge on their surface and according to the charge of the amino acid present in them .if they positive,they will migrate to the negative pole (the cathode) .

The polymerase chain reaction (PCR) , which is a laboratory cloning technique , was performed to amplify a region of DNA whose sequence is known or located between two regions of known sequence.

The primer pairs, ITS5 and ITS4 was adopted, which is a general pair that targeted the location of the fungal gene sequence table(1). , and a mixture was formed with a final volume of 25 microliters table(2).

Table (1) Primers used in the experiment

Primer	Sequences(5`-3`)	size	Reference
Universal primer	ITS5: GGAAGTAAAAGTCGAACAAGG ITS4: TCCTCCGCTTATTGATATGC	500-850bp	Bellemain, *et a.,*/ 2010

Table (2)chemicals for the reaction

chemicals	Volume/tube
Master mix promega	12microliter
Primer forward	1.2 microliter
Primer reverse	1.2 microliter
DNA	1 microliter
D.W	9 microliter
Total	25 microliter

The isolated replication results from the PCR reaction were sent to Macrogen Company in south Korea for the purpose of determining the sequence of nitrogenous bases. The nitrogen sequences were analyzed using the BLAST program(Basic Local Alignment Search Tool) in order to find out the similarity between the studied pathogenic fungi and the globally registered fungi and compared them with data recorded in the Gene Bank of the information center Biotechnology NCBI(National Center For Biotechnology information) (7), and the evolutionary chart of the species was drawn on the basis of the Mega 6 program.Figure (2),(3)and(4)

Ethical approval: The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patients verbal and analytical approval before sample was taken. The study protocol and the subject information and consent form were reviewed and approved by a local ethics committee according to the document number 4347 in 16-12-2021

Result

The region of the marker molecule ITS was amplified by adopting the Universal primes ITS5 and TTS4 of tow isolate from dermatophytes and one isolate from Candida yeast figure(1)

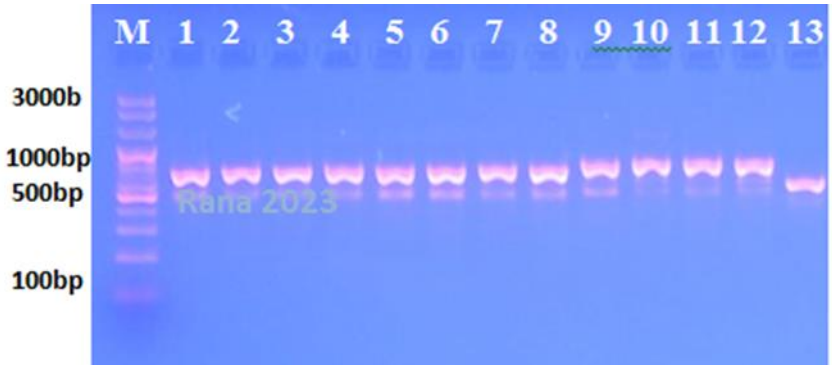

Figure (1) Electrophoresis on agarose gel 1.5% and 100 Voltag for one hour and Marker Ladder (100-3000 bp),10= T.indotineae,12= T.qunickeanum(13 = C.albicans ,1,2,3,4,5,6,7,8,9,11 other aisolate

Table (3) Sequence cods identified in the current bank for isolated pathogenic

Serial code	Species
OQ999337.1	*T.indotineae*
OR083657.1	*T.qunckeaium*
OR085961.1	*C.albicans*

Table(4) comparison of the sequence of the nitrogenous bases of the ITS region of the T.indodtineae fungus isolated in this study with other isolates of the same fungus recorded

Mach ratio	origon	strain name	serial code
*	Iraq	*T. indotineae*	OQ999337.1
99.25	Iraq-Baghdad	*T. indotineae*	MT371234.1
99.25	Iraq-Baghdad	*T. indotineae*	MT3712233.1
99.25	Germany	*T. indotineae*	MT333228.1
99.25	Finland	*T. indotineae*	MN661259.1
99.25	Germany	*T. indotineae*	MT333225.1
99.25	Germany	*T. indotineae*	MH791418.1
99.25	Germany	*T. indotineae*	OM951144.1
99.25	Germany	*T. indotineae*	MH791424.1
99.25	Germany	*T. indotineae*	MT330253.2

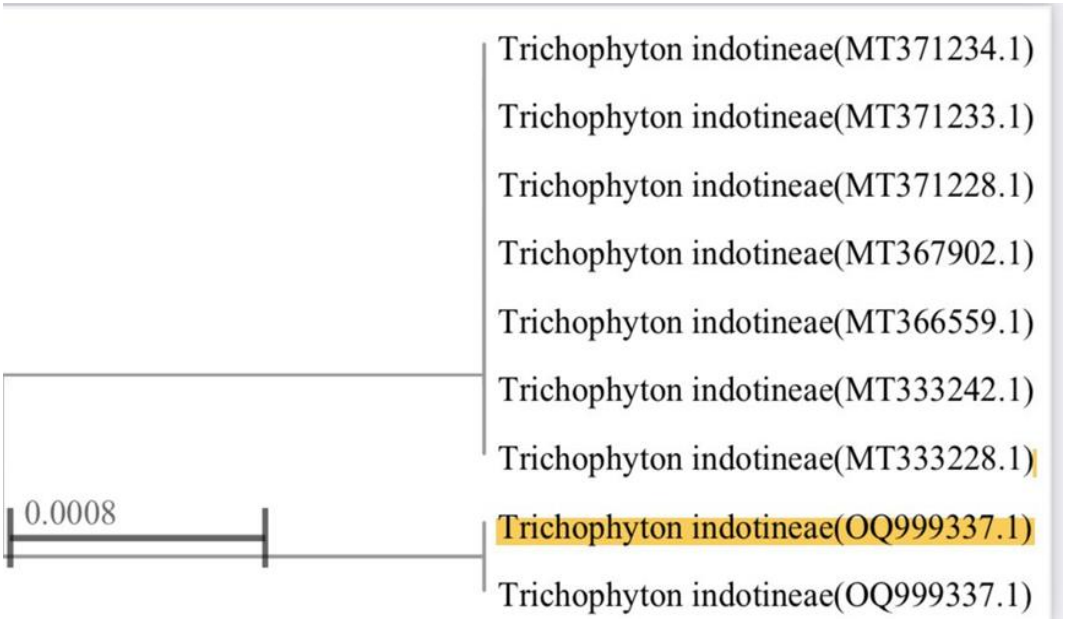

Figure (2) the genetic tree of the fungus T.indotineae highlighted in yellow,shows the relationship between the global fungal strains

Table(5) comparison of the sequence of the nitrogenous bases of the ITS region of the T.quincke

Mach	Origon	Strain	Serial code
*	Iraq	*T.quinckeanum*	OR083654.1
95.37	Iraq	*T.quinckeanum*	OQ979292.1
98.80	Belgium	*T.quinckeanum*	OW984761.1
98.80	USA	*T.quinckeanum*	KJ606088.1
98.81	Iran	*T.quinckeanum*	MN808779.1
100	Iraq	*T.quinckeanum*	0P821484.1
100	Iraq	*T.quinckeanum*	0P4195960.1
99.11	Iran	*T.quinckeanum*	OP391642.1

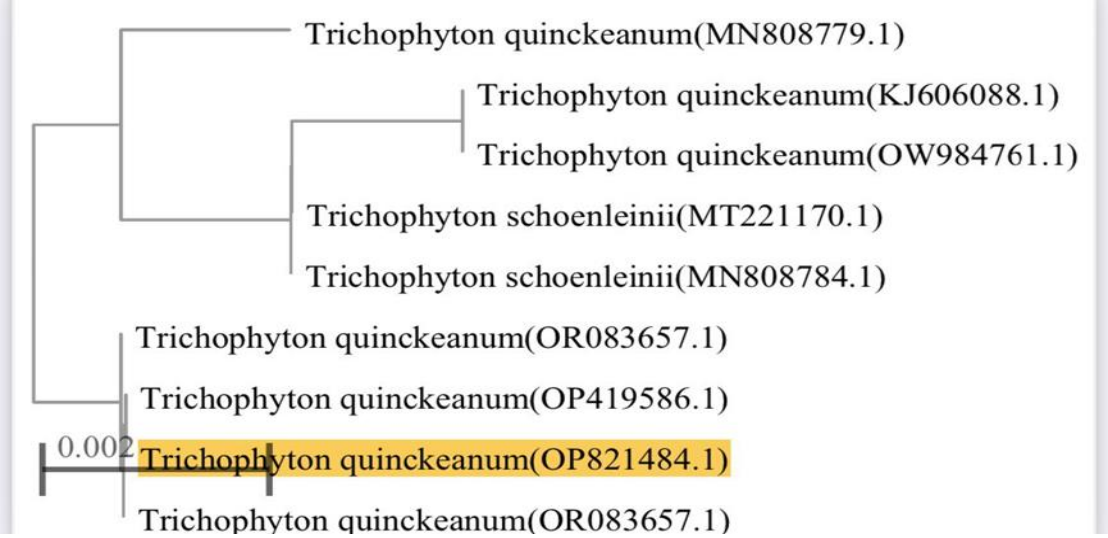

Figure (3) the genetic tree of the fungus T.quinckeanum highlighted in yellow,shows the relationship between the global fungal strains.

Table(6) comparison of the sequence of the nitrogenous bases of the ITS region of the C.albicans fungus isolated in this study with other isolates of the same fungus record.

Mach	Origon	Strain name	Serial code
*	Iraq	*C.albicans*	OR08591.1
98.51	Saudi arabia	*C.albicans*	MN419373.1
98.51	Saudi arabia	*C.albicans*	MN419378.1
98.51	Saudi arabia	*C.albicans*	MK3077501.1
99.06	Saudi arabia	*C.albicans*	MK568486.1
99.25	Saudi arabia	*C.albicans*	MN419377.1
99.0	Saudi arabia	*C.albicans*	MN419364.1
99.0	Saudi arabia	*C.albicans*	MK560180.1

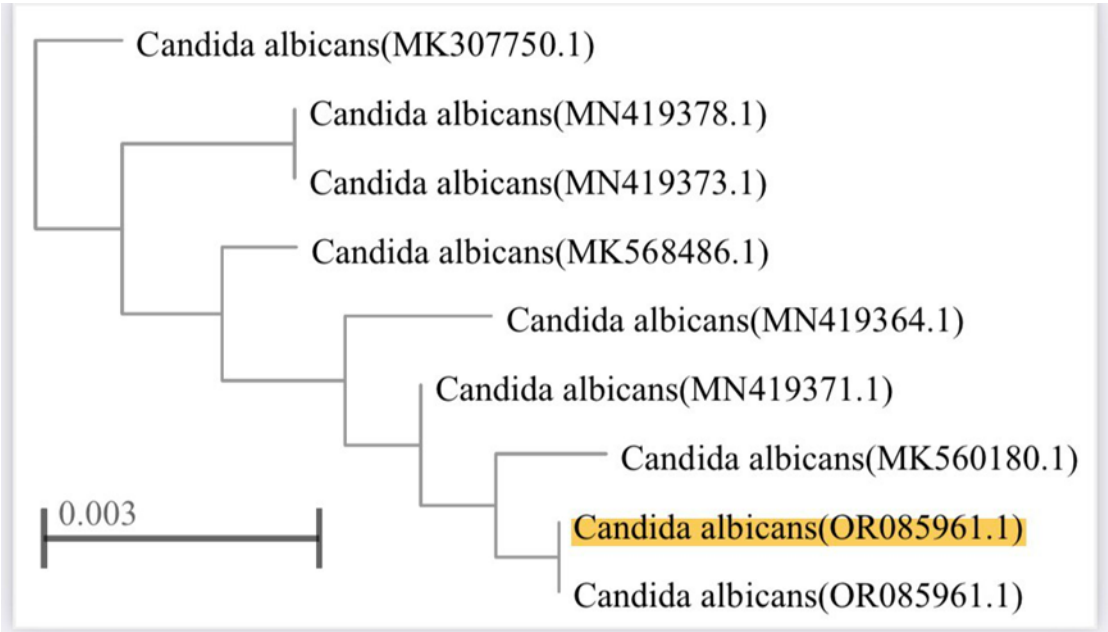

Figure (4) the genetic tree of the fungus C.albicans highlighted in yellow,shows the relationship between the global fungal strains.

Discussion

The result of the polymerization showed unique bands that ranged between 510-850 base pair .this is identical to the result of a study (8) that used the general primer pair ITS4 and ITS5 to amplify ITS in the Candida , which gave individul size ranged between 510-850 , .indotineae(690bp), T. qunickeanum (700bp) , C.albicans (550bp) figure(1).the results of the polymerization of some of the studied cutaneous fungal isolates agree with the results of (9),where the size of the polymerization ranged between 680-700base pairs , while the size of Candida yeast bundle was 550 base pairs.

The genetic tree is used to find out the association of all species of a particular genus with the species to be identified , in addition to the phenotypic characteristics and traits, and to obtain an accurate diagnosis in addition to the traditional diagnosis .Determining the genotype is important in the classification of fungi and enhances the initial diagnosis (10)(11) The result of the analysis of the nitrogenous bases of the doubled nucleic acid products using the Blast program proved that the studied isolates belong to the species: T.indotineae,T.qunickum, C.albicans

It became clear by comparing the sequence of nitrogenous bases that there is a match between the isolates that were used in the study and the global isolates. This was confirmed by the results of the phylogenetic analysis , which showed that the fungal isolate was combined with groups consisting of isolates belonging to the same species , by drawing the genetic tree. Genetic distance was calculated using the neighbor - joining method , and the evolutionary genetic analysis program MEGA V. 11 was used , which is one of the program designed to compare and analyze the sequence of similar genes ,the pattern of DNA and protein development ,and the evolutionary relationships. It is possible to display this date in the form of an evolutionary tree)12.(

In the table(4),it was found that the study isolate T. indotineae showed a genetic similarity of 99.25 with each of Iraq-Baghdad, Germany and Finland).

In the table (5) ,it was found that the study isolate T.quinckeanum showed a genetic similarity of 95.37 with another isolate in Iraq , 98.80 with Belgium and USA , 98.81 with an isolate in Iran , 100 with two another isolate in Iraq and 99.11 with another isolate in Iran

In the table (6)it was found that study isolate C.albicans showed similarity with several isolates in Saudi arabia in different proportions ,as it was 98.51 with three isolates ,99.06 with one isolate ,99.25 with another and 99.0 with two other isolates.

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