Isolation and Diagnosis of Some Dermatophytes Using Nitrogen Base Tracking Technique (Scquence)

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Abstract

A total of, 162 Samples of those infects with dermatophytes at lmam Hussein Teaching Hospital in Nasiryah city for the purpose of diagnosing some samples in a sequential manner , The samples included skin skimmers, nail clippers, hair pieces, Isolation and diagnosis of samples was carried out by conventional methods and then confirmed the diagnosis in a way that follows the nitrogen bases, where 10 samples were diagnosed and recorded in the gene bank , Trichophyton was the more frequent , than Epidermophyton ,A number of Candidia yeasts were also diagnosed in the traditional methods of appearance , It was a ratio of T. rubrum (28%) than T. interdigitale (24%), than T. mentagrophytes (21%), and also E. floccosum in the rate of (25%) , There are also ratios for the emergence of types of yeasts as a proportion C. albicans (32%) , Than C. parapsilosis (25%) , Than C. kruzi (22%) , and also C. dubllirensis in the rate of (19%) , Thes samples were collected from different ages and from both sexes .

Key words : *Dermatophytes, Nitrogen base tracking technique (Scquence).*

Introduction

Dermatophytes are a group of fungi that have the ability to attack keratinized tissues of humans and animals such as skin, hair and nails, causing dermatophytes, Thes fungi comprise three species (Trichophyton Epidermophyton, Microsporu) [1] .

Trichophyton is characterized by the formation of large conidates and small conidaes with a smooth wall and this genus includes a number of species that have the ability to infect the skin, hair and nails [2] .

Skin infections are a common infection in humans for a long time as millions of people in the world are exposed [3] , It causes skin infections known as ringworm(Tineas) or ringwor infection (Tinea) or skin mycosis in humans and animals [4] , It has the advantages of being a keratin-loving keratin (Keratinophilic) and his analyzer (Keratinolytic), but has no ability to penetrate deep tissus below the keratatinized layer as most of them are unable to live at high temperature such as body temperature [5] .

The incidence rates vary depending on the location of the infection in the body and age, as the incidence of scalp (Tinea capitis) in younger ages is more than in adults , Asat [6] and his group in 1996 and the researcher (Chin,2000) [7] reported that the relative resistance to infection is due to the long chains of saturated fatty acids found in sebum produced after puberty , ringworm (Tinea corporis) occurs in warm areas of the world and affects more children, especially those in direct contact with animals and adults, especially in those with excessive sweating [8] , While ringworm (Tinea pedis) affects athletes in general because of its spread in swimming pools and clothing strikes, and crowded and public place (such as schools, hospitals, and public parks) are preferred sites for the emergence and wide variety of pathogenic and opportunistic fungi .

There are many laboratory methods used to diagnose skin fungi [9] one of these methods is forming on food circles [10] ,There are also special circles used to diagnose skin fungi called Dermatophytes Identification Media (DIM) in these settings, the diagnosis depeds on the color variation of the fungal colonies [11] , Diagnosis can also be made based on specific nutritional requirements for the growth of fungi, such as the need for the thymine compound T. violaceum , [12] Currently, the PCR technique is used to diagnose fungi if the fungal colony lacks the diagnostic phenotypic characteristic or

the newly developed fungal colony or if the fungus is dead [13] .

Materials and Method

Collect samples

About 162 samples (hair pieces, demabrasion , and nails) were collected from patients attending lmam Hussein Teaching Hospital in the Dermatology Consultation Department in Thi-Qar Governorate. (Gender, age, area of residence, injury area as well as the work of the injured person and date of sample collection) .

Examination of samples

Direct microscopy of samples The samples were transferred to the college laboratory as they were taken from the affected skin, hair and nails and added drops of potassium hydroxide solution at a concentration of 10% after being placed on a glass slide and left for 5 minutes of time . ln microscopic examination using a complex optical microscope and at a power of (40X) .

Cultivation of specimens

The remaining part of the samples is planted on the medium of Sabouraud Dextrose Agar with Cycloheximide and Chloramphenic . The medium of Sabouraud Dextrose Agar added whith Cycloheximide and Chloramphenic to prevent the growth of opportunistic bacteria and fungi , Prepare the medium by melting 65 g of sabouraud dextrose agar in 1000 ml distilled water , sterilize with anodized and cool to 45 C and add 0.05 of chloramphenic antibiotic to inhibit bacterial growth and 5.0 cycloheximide to inhibit the growth of unsatisfactory thrush , use this medium to isolate grow and preserve fungal isolates [14] .

Diagnosis

Cultivated dishes were examined five days after transplantation and then left for three weeke at 28 C to allow more fungi to grow and appear , the fungi were then isolated from dishes containing SDA medium , the isolates are then purified and transferrwd from the colonial parts to the slanted media , it was incubated at 25 °C and then kept in the refrigerator at 4 °C to 6 °C until later use . All fungi were laboratory diagnosed based on the cultivative and phenotypic qualities and with the help [15,16,17] .

DNA sequencing method

DNA sequencing method was performed for species typing of positive Trichophyton sp. isolates as following step:

1- The PCR product of 18S ribosomal RNA genes were sent to Macrogen Company in Korea in ice bag by DHL for performed the DNA sequencing by AB DNA sequencing system.

2- The DNA sequencing analysis (Phylogenetic tree analysis) was conducted by using Molecular Evolutionary Genetics Analysis version 6.0. (Mega 6.0) and Multiple sequence alignment analysis based ClustalW alignment analysis and The evolutionary distances were computed using the Maximum Composite Likelihood method by phylogenetic tree UPGMA method.

3- The Trichophyton species typing analysis was done by phylogenetic tree analysis between local Trichophyton sp. isolates and NCBI-Blast known Trichophyton species.

4- Finally identified Trichophyton species isolates were submitted into of NCBI-GenBank to get Genbank accession number .

Medium Chrome Agar Candida

Ues to distinguish between the types of Candida , prepare the medium according to the instructions of the manufacturer by adding the powder medium (47 g) slowly to sterile distilled water Balmsdah (1000 ml) with stirring to homogenize the mixture and then heat to 100 C until large bubbles appear then cool to a degree 50 °C water bath and poured into Petri dishes , then samples were planned on the surface of the medium and incubated at 37 °C and you monitor growth for (24-48) hours and then observed the change in the colors of the colonines growing on this medium [18] .

Results and Discussion

The results of laboratory transplantation on the medium of Sabouraud Dextrose Agar showed the presence of skin fungi in 70 samples of the total samples as shown in Table (1) , While the number of samples in which Candida appeared was 40 samples as shown in Table (2) , as for the results of the laboratory examination, 30 samples showed dermatophytes , Candida samples were 22, as shown in Table (3) , The

results of this table indicate that direct microscopic examination is highly sensitive in the diagnosis of skin injuries compared with implantation on the media [19] They pointed out that the direct examination as a detection of the presence or absence of fungi , The emergence of negative results, whether in direct microscopy or laboratory transplantation for many reasons, the most important inaccurare diagnosis as the infection is not fungal, but are symptoms of allergic diseases, and also some patients resort to the use of treatments without consulting a doctor, which leads to influence the activity of the fungus causing the infection [20] , and not grow until transplantation [21] .

Table (1) number of fungal isolates isolated from skin, hair and nails

Mushroom type	The number	Mean%
T. mentagrophytes	15	21
T. interdigitale	17	24
T. rubrum	20	28
E. floccosum	18	25

The total number of positive isolated fungi (70) was calculated by the following equation

Number ÷ Total number × 100)))

Table (2) number of candida isolates

Candida type	The number	%Mean
C. albicans	20	32
C.dublliensis	12	19
C.parapsilosis	16	25
C. kruzi	14	22

The total number of isolated Candida was (62) and the percentage was calculated by the following equation

Number ÷ Total number × 100))))

Table (3) Results of microscopy and transplantation of samples

Type of examination	Dermato-phytes	Percen-tage	Candida	Mean%
Direct microscopy (KOH)	30	30%	22	22
Fungal transplants results	70	70%	40	40

The study showed the diagnosis of three species of the genus Tricchophyton from the samples included in the study which T. mentagrophyte and T. rubrum and T. interdigitale with one species of the genus Epiderophyton which is E. floccosum on the medium of Sabouraud Dextrose Agar added whith Cycloheximide and Chloramphenic , the study also showed the emergence of types of genus Candida , namely C. albicans and C. dubelliensis and C. parapsilsis with C. kruzi it was grown on the medium Chrom Agar as shown in the following figures :

Molecular diagnosis using polymerase chain reaction

Nucleic acid was extracted from the isolates under study and the result was the emergence of DNA bundles clearly when imaging with UV light and for all the isolates selected using electrophoresis technique on acarose gel as shown in Figure (1) .

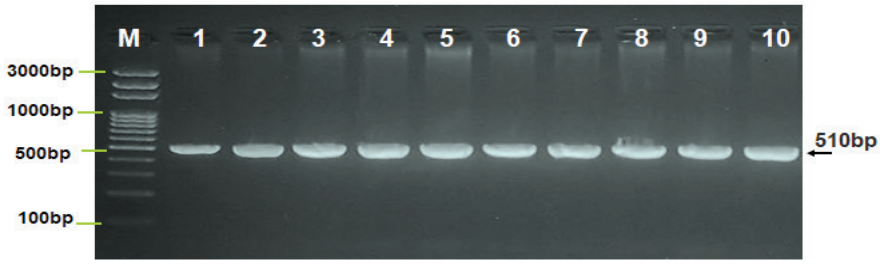


Figure (1) represents the shape of shape of DNA produced using PCR technology

The results of the sequence for a set of studied samples

Ten specimens in the present study showed the new species of the genus Trichophyto sp . that were registered at the lnternational NCBI- Genbank, the results revealed that there were many variables in many of the samples examined compared to the DNA sequence recorded in the bank .

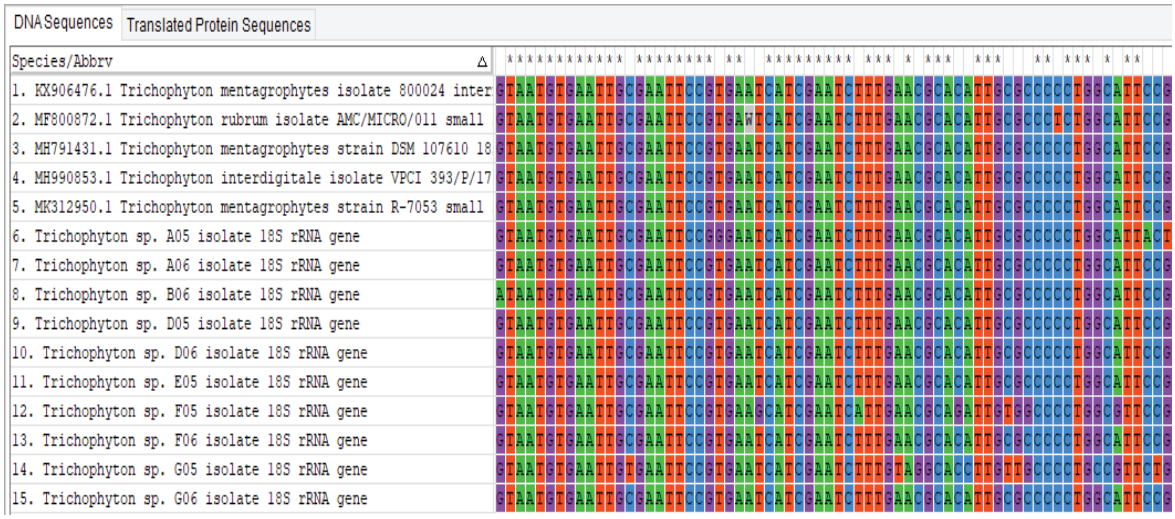


Figure (2): Multiple sequence alignment analysis of18S rRNA gene in local Trichophyton sp.isolates and NCBI-Genbank Trichophyton sp. Isolates. The multiple alignment analysis was constructed using ClustalW alignment tool in (MEGA 6.0 version). That show the nucleotide alignment similarity as (*) with substitution mutations in 18S rRNA gene.

Trichophyton mentagrophytes isolate IQ-E5 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence

GenBank: MN165773.1

FASTA Graphics

Go to:

LOCUS MN165773 324 bp DNA linear PLN 16-JUL-2019

DEFINITION Trichophyton mentagrophytes isolate IQ-E5 internal transcribed

spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete

sequence; and internal transcribed spacer 2, partial sequence.

ACCESSION MN165773

VERSION MN165773.1

KEYWORDS .

SOURCE Trichophyton mentagrophytes

ORGANISM Trichophyton mentagrophytes

Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina;

Eurotiomycetes; Eurotiomycetidae; Onygenales; Arthrodermataceae;

Trichophyton.

REFERENCE 1 (bases 1 to 324)

AUTHORS Abood,M.S. and Naser,S.K.

TITLE Study on some genes associated with azole antifungal resistance of

Dermatophytes

JOURNAL Unpublished

REFERENCE 2 (bases 1 to 324)

AUTHORS Abood,M.S. and Naser,S.K.

TITLE Direct Submission

JOURNAL Submitted (09-JUL-2019) Biology, College of Education for Pure

Science/University Of Thi-Qar, Nasiriyah, Nasiriyah, Thi-Qar 00964,

Iraq

COMMENT ##Assembly-Data-START##

Sequencing Technology :: Sanger dideoxy sequencing

##Assembly-Data-END##

FEATURES Location/Qualifiers

source 1..324

/organism="Trichophyton mentagrophytes"

/mol_type="genomic DNA"

/isolate="IQ-E5"

/isolation_source="skin lesion"

/host="Homo sapiens"

/db_xref="taxon:523103"

/country="Iraq"

misc_RNA <1..>324

/note="contains internal transcribed spacer 1, 5.8S

ribosomal RNA, and internal transcribed spacer 2"

ORIGIN

1 tttcagagat gtgtctgtcg gagatttagt agtgaaaatc agttaaaact ttcgacaacg

61 gaattctggg ttccggcatc gatgaagaac gcagcgaaat gcgataagta atgtgaattg

121 cagaattccg tgaatcatcg aatctttgaa cgcacattgc gccccctggc attccggggg

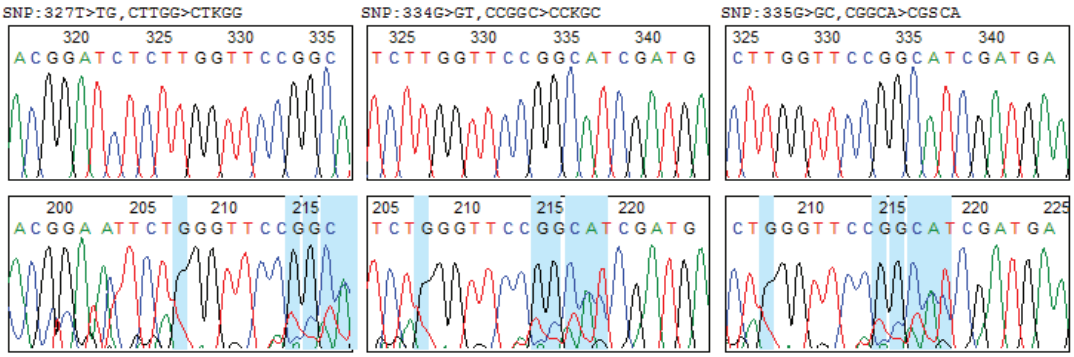
181 gcatgcctgt tcgagcgtca tttcagcccc tcaagcccgg cttgtgtgat ggacgaccgt

241 ccggcgcccc cgtttttggg ggtgcgggac gcgcccgaaa agcagtggcc aggccgcgat

301 tccggcttcc taggcgaatg ggca

This fungus registered Trichophyton mentagrophytes as a local isolation in the gene bank with assession number MN165773

E05



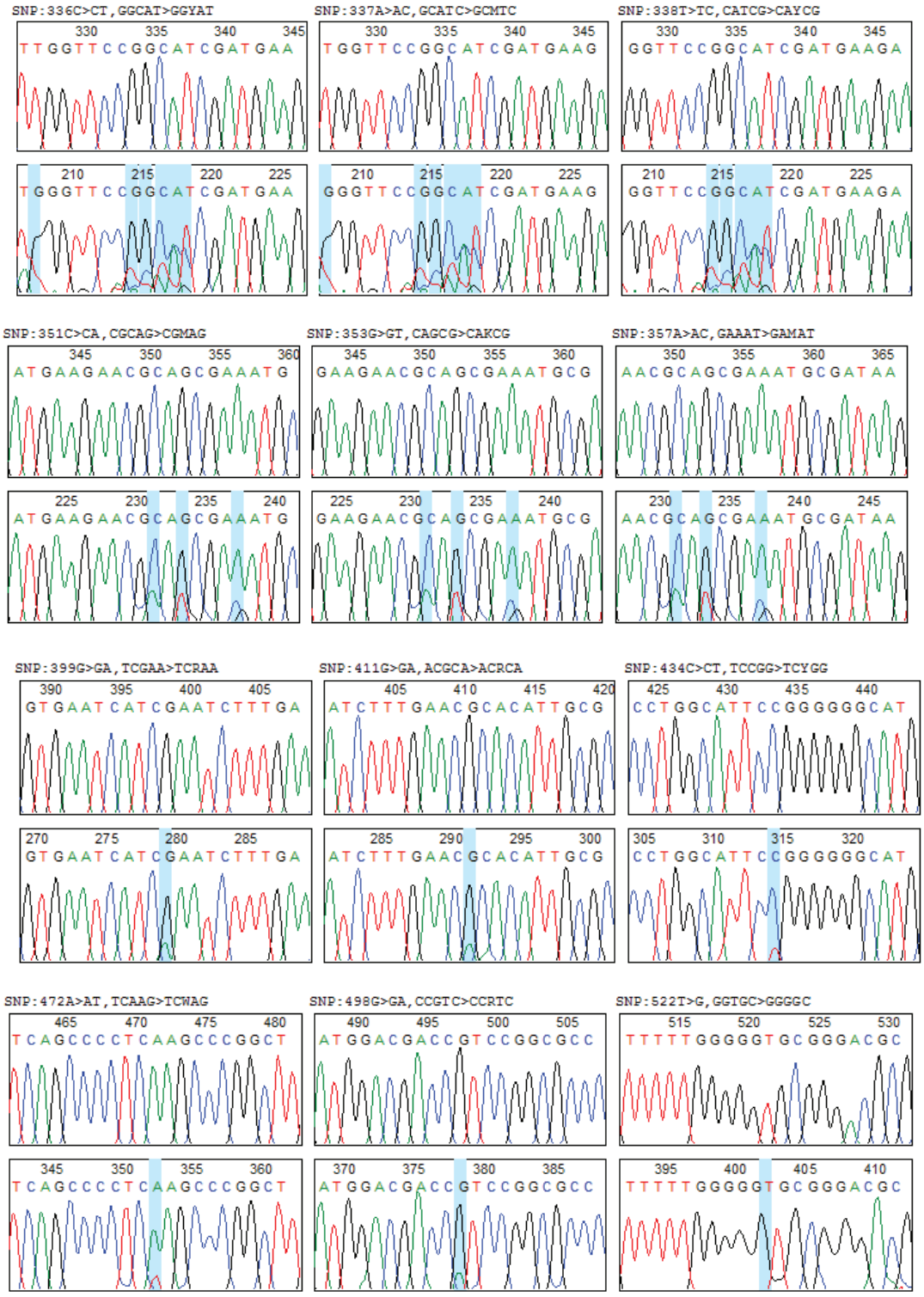


Figure (3): Treatment of the sequence of nucleotides of the isolated local Trichophyton mentagrophytes

Table (4): the NCBI-BLAST Homology Sequence identity (%) between local Trichophyton spisolates and NCBI-BLAST submitted Trichophyton sp. isolates:

NCBI-BLAST Homology Sequence identity (%)			Genbank Accession number	Trichophyton sp. Isolate No.1
Identity(%)	Genbank Accession number	Identical Trichophyton sp.		
91.88%	MK312950.1	Trichophyton mentagrophytes	MN165768	Tricophyton sp. A05 isolate
99.62%	MH791431.1	Trichophyton mentagrophytes	MN165769	Trichophyton sp. A06 isolate
94.69%	MH791431.1	Trichophyton mentagrophytes	MN165770	Trichophyton sp. B06isolate
91.70%	KX906476.1	Trichophyton mentagrophytes	MN165771	Trichophyton sp. D05 isolate
94.77%	MH791431.1	Trichophyton mentagrophytes	MN165777	Trichophyton sp. D06 isolate
95.40%	MH791431.1	Trichophyton mentagrophytes	MN165773	Trichophyton sp. E05 isolate
85.79%	KX906476.1	Trichophyton mentagrophytes	MN165774	Trichophyton sp. F05 isolate
97.01%	KX906476.1	Trichophyton mentagrophytes	MN165775	Trichophyton sp. F06 isolate
80.41%	MF800872.1	Trichophyton rubrum	MN165776	Trichophyton sp. G05 isolate
92.64%	MH791431.1	Trichophyton mentagrophytes	MN165772	Trichophyton sp. G06 isolate

The reliance on molecular analysis of DNA sequences in the present study to diagnose dermatophytes is of great importance in supporting phenotypic diagnostic methds

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the University of Thi-Qar- College of Education for Pure Sciences and all experiments were carried out in accordance with approved guidelines.

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