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Original Article

Molecular characterisation of *Malassezia* species isolated from healthy individuals and patients with Pityriasis Versicolor in the Thi-Qar Governorate of Iraq

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Abstract

Background On the skin of humans and other warm-blooded animals, lipophilic yeasts called *Malassezia* dwell in a symbiotic relationship. There are two types of yeast pseudofilamentous yeast and unicellular yeast. *Malassezia* species cause pityriasis versicolor by invading the skin's outer keratinized layer, resulting in patch development and coloring.

Objective To molecularly describe isolates of *Malassezia* from both healthy people and sufferers.

Methods Between July and December 2023, 30 skin swabs from healthy people and 72 skin scraping samples from patients with pityriasis versicolor were obtained. DNA sequencing and PCR were used to evaluate the samples.

Results Significant variation was seen in the *Malassezia* isolates from both sick and healthy people, according to genetic research. Several mutations were identified, and new strains were registered in GenBank.

Conclusion There is a strong genetic relationship between pathogenic and control isolates, with high genetic diversity observed among *Malassezia* species.

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Introduction

Malassezia species, formerly known as *Pityrosporum*, are opportunistic lipophilic yeasts that naturally inhabit human skin.[1] Under favorable conditions, they shift from a yeast form to a pathogenic hyphal form, causing superficial or systemic infections.[2] These yeasts belong to the phylum Basidiomycota and include approximately 17 specie.[3]

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While *Malassezia* typically contributes to skin barrier integrity and immune balance[4] it is also implicated in various dermatological conditions such as pityriasis versicolor, seborrheic dermatitis, dandruff, psoriasis, and steroid acne.[5]

Pityriasis versicolor is a chronic superficial fungal infection characterized by multicolored patches on the skin, commonly affecting the face, neck, chest, and back.[6] It is more prevalent in hot and humid climates and among young individuals. Although non-contagious, it is associated with genetic predisposition, hyperhidrosis, and immunosuppression.[7]

Due to their lipophilic nature *Malassezia* species

colonize sebaceous rich areas of the skin and are more prevalent in adults than infants.[8] Their pathogenicity is linked to the production of enzymes such as lipases, proteases, esterases, phospholipases, and lipoxygenases, which contribute to inflammation and irritation in affected areas.[9]

Despite the global relevance of *Malassezia* species in dermatological conditions, molecular studies in Iraq particularly in the southern region remain scarce. This study to investigate the genetic diversity and molecular characteristics of *Malassezia* species isolated from patients with pityriasis versicolor and healthy individuals using PCR and DNA sequencing techniques.

Methods

This study was designed as a case-control study. Seventy-two skin scrapings from clinically diagnosed pityriasis versicolor patients and 30 swabs from healthy individuals (The sample distribution was unequal due to the limited availability of eligible participants during the study period) were collected from dermatology clinics in AL-Nasiriyah, AL-Hussein, and AL-Shatra hospitals between July and December 2023 (skin scrapings were collected from active lesions of patients to ensure adequate fungal load for molecular detection. In contrast, skin swabs were obtained from healthy individuals to avoid unnecessary invasive sampling, as no visible lesions were present. This approach was adopted in accordance with ethical considerations and standard dermatological sampling practices). Informed consent was obtained from all participants before sample collection in accordance with ethical research standards. Inclusion criteria for patients included a clinical diagnosis of pityriasis versicolor and absence of systemic antifungal therapy within four weeks prior to sampling**.** Exclusion criteria included patients with chronic systemic diseases, immunosuppressive conditions, or recent antifungal treatment**.** Healthy controls had no history of dermatological disease or recent antifungal therapy. These clinics included AL-Nasiyriah, AL-Shatra, AL-Graph, AL-Nasser, AL-Rifaei, Qaleat Sukar, and AL-Aslah **(Figure 1)**. Samples were examined microscopically with 10% KOH, cultured on Sabouraud dextrose agar with and without olive oil, and incubated at 30°C for 5-10 days.[10-13] DNA was extracted using the Geneaid™ Yeast DNA Isolation Kit and amplified with *26S rDNA* and *β-tubulin* gene primers.[14,15] DNA amplification was performed using two nuclear targets, *26S rDNA* and *β-tubulin*, for sixty *Malassezia* isolates in a final PCR reaction volume of 25 µl **(Table 1).** For the *26S rDNA* target, the primers used were MalF (5′-TAACAAGGATTCCCCTAGTA-3′) and MalR (5′-ATTACGCCAGCATCCTAAG-3′). The PCR cycling conditions consisted of an initial denaturation at 95°C for 5 min, followed by 32 cycles of denaturation at 95°C for 45 sec, annealing at 55°C for 45 sec, and extension at 72°C for 1 min, with a final extension at 72°C for 7 min.[16]

For the *β-tubulin* target, the primers used were β-tubF (5′-CARGCYGGTCARTGYGGTAACCA-3′) and β-tubR (5′-GCCTCAGTRAAYTCCATYTCRTCCAT-3′). The PCR reaction conditions included an initial denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 95°C for 30 sec, annealing

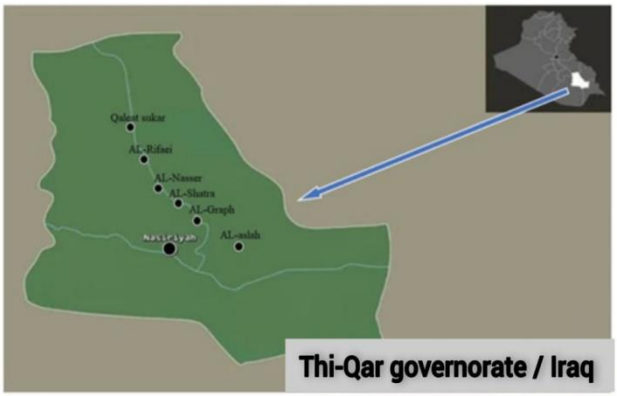

Figure 1 Sampling sites on Thi-Qar province/ southern of Iraq.

Table 1 The Mixture of PCR Working Solution

No.	*Components*	Volume (µl)
1-	DNA template (30-100ng)	3 µl
2-	Primer forward (Mal & β-tub)	1 µl
3-	Primer reverse (Mal & β-tub)	1 µl
4-	Master Mix	13 µl
5-	Free nuclease water	7 µl
Total 25 µl		

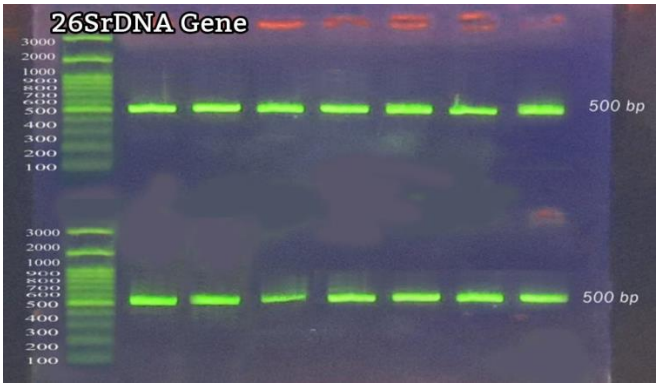

Figure 2 Agarose Gel Electrophoresis of 26S rDNA Gene with 500bp size (1.5% agarose , TBE ,70 Volte, 85 mA , 45 min).

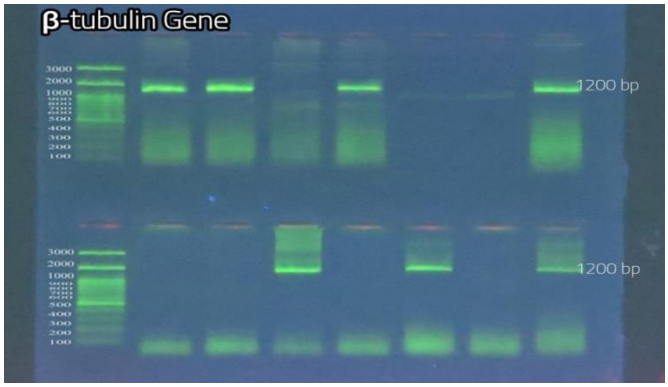

Figure 3 Agarose Gel Electrophoresis of β-tubulin Gene with 1200bp size (1.5% agarose , TBE ,70 Volte, 85 mA , 45 min).

at 50°C for 1 min, and extension at 72°C for 1 min, with a final extension step at 72°C for 10 min.[17] Gel electrophoresis of the PCR products was performed on a 1.5% agarose gel to verify successful gene amplification.[3] Sequencing was performed by Macrogen (Korea), and sequences were analyzed using BLAST and MEGA X for phylogenetic analysis.[18] As the main objective of this investigation was molecular identification and genetic characterization of isolates, the results were interpreted descriptively without inferential statistical analysis.

Results

The Mal primer successfully amplified the *26S rDNA* gene (D1/D2 region), producing 60 PCR products with an approximate fragment size of 500 bp **(Figure 2).**

In contrast, the β-tub primer successfully amplified the *β-tubulin* gene from 38 DNA isolates, yielding fragments of about 1200 bp**. (Figure 3).**

The results obtained from the BLAST tool on the NCBI GenBank website revealed that 25 isolates belonged to Malassezia furfur and 4 isolates belonged to *Malassezia globosa***.**

All *Malassezia* sequences were deposited in the GenBank database using the BankIt submission tool under specific accession numbers **(Table 2).**

Table 2 Matching between the isolates under study and global isolates registered in NCBI

No. Sample	*Scientific Name*	*Accession Number (for this study)*	*Identification %*	*Reference Copies (NCBI)*	*Query cover %*
1	Malassezia globosa	PP989341	100%	CP046435.1	100%
2	Malassezia furfur	PP889897	99.62%	MT211523.1	100%
3	Malassezia globosa	PP989342	100%	CP046435.1	100%
4	Malassezia furfur (Control)	PP889898	99.81%	MT211523.1	100%
5	Malassezia furfur	PP889899	100%	MH294226.1	100%
6	Malassezia globosa (Control)	PP889900	100%	MH294247.1	100%
8	Malassezia furfur (Control)	PP889901	100%	MH294226.1	100%
9	Malassezia globosa	PP989343	98.85%	MW007972.1	100%
11	Malassezia furfur	PP889902	100%	CP046435.1	100%
12	Malassezia furfur	PP889903	99.31%	CP046435.1	100%
13	Malassezia furfur	PP889904	99.81%	MH294247.1	100%
14	Malassezia furfur	PP889905	99.54%	CP046435.1	99%
15	Malassezia furfur	PP889906	100%	CP046435.1	100%
16	Malassezia furfur	PP889907	100%	CP046435.1	100%
17	Malassezia furfur	PP889908	100%	CP046435.1	100%
18	Malassezia furfur	PP889909	99.57%	CP046435.1	100%
19	Malassezia furfur	PP889910	99.79%	MT211538.1	100%
20	Malassezia furfur	PP889911	99.94%	CP046435.1	100%
21	Malassezia furfur	PP889912	99.57%	CP046435.1	100%
22	Malassezia furfur	PP889913	99.15%	CP046435.1	100%
23	Malassezia furfur	PP889914	100%	MH294247.1	100%
24	Malassezia furfur	PP889915	99.78%	CP046435.1	99%
25	Malassezia furfur	PP889916	99.77%	MH294243.1	100%
26	Malassezia globosa	PP989344	99.56%	CP046435.1	96%
27	Malassezia furfur	PP889917	99.73%	MH294247.1	100%
28	Malassezia furfur	PP889918	100%	MT000716.1	100%
29	Malassezia furfur	PP889919	99.76%	CP046435.1	100%
30	Malassezia furfur	PP889920	99.47%	CP046435.1	100%
31	Malassezia furfur	PP889921	99.75%	CP046435.1	100%

A total of 25 *Malassezia* furfur isolates were used to construct a phylogenetic tree using the Maximum Likelihood method based on the Tamura-Nei model implemented in MEGA X software**.**

Multiple sequence alignment analysis of the *26S rDNA* gene demonstrated genetic variation among the local isolates and reference strains from the GenBank database, caused by substitution mutations**.**

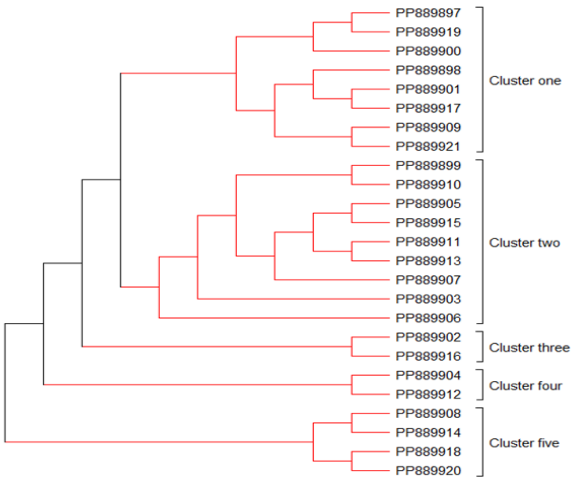

Figure 4 Phylogenetic tree constricted using the Maximum Likelihood method based on the Tamura-Nei model as implemented in Mega X software showing five genetic groups (Clusters) of 25 Malassezia furfur isolated from clinical samples.

A considerable degree of genetic variety among the *Malassezia* species in this study was indicated by the phylogenetic tree, which showed four unique groups**.**

Nine isolates were found in Cluster II, eight in Cluster I, four in Cluster V, and two in each of Clusters III and IV **(Figure 4).**

Substitution mutations between the local and reference isolates were also found by multiple sequence alignment analysis of the *26S rDNA* gene **(Figure 5).**

A number of changes that happened in the *Malassezia* DNA sequences when compared to the reference strains are shown in **Figure 5**. A nucleotide substitution occurred at site 51, where base A in the reference sequence changed to G in isolates PP889898 and PP889904, and at site 67, where base G changed to T in isolates PP889906,

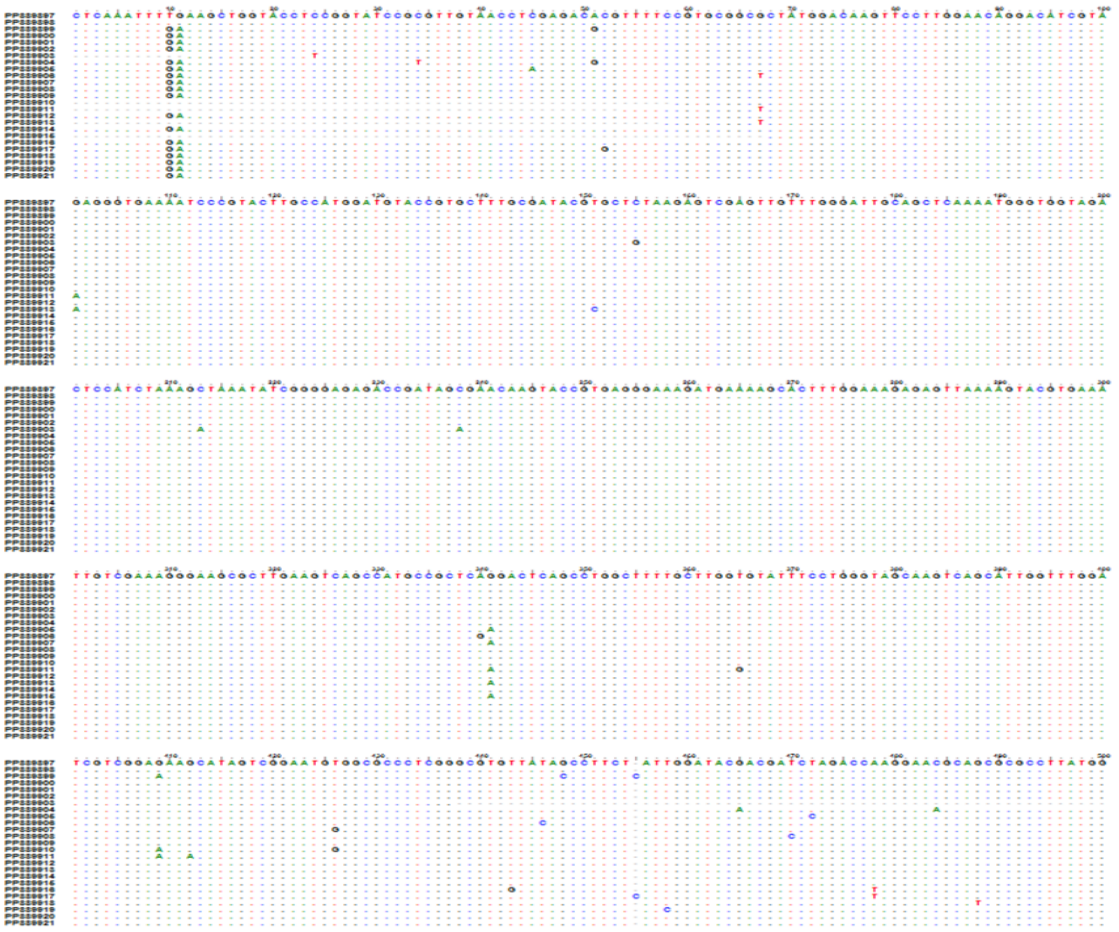

Figure 5 Multiple alignment sequence analysis of the DNA of the isolates under study and letters indicate (A, T, C, G) nucleotide bases and dots indicate positions where sequences are identical to the reference sequence.

PP889911, and PP889913**.** At site 1, base G in the reference sequence changed to A in isolates PP889911 and PP889913, while at site 55, base C changed to G in isolate PP889903**.**

Base C changed to A at sites 13 and 38 in the same isolate, and base T changed to G at site 65 in isolate PP889911**.** Additionally, base A changed to G at site 40 in isolate PP889906**.**

At site 48, base G in the reference sequence changed to C in isolate PP889899, and at site 26, base T changed to G in isolates PP889907 and PP889910.

Discussion

Molecular identification using the *26SrDNA* (D1/D2 region) and *β-tubulin* gene targets provided reliable and accurate differentiation among closely related *Malassezia* species in the present study. The predominance of *Malassezia furfur* among clinical isolates indicates that this species represents the principal etiological agent of pityriasis versicolor in Thi-Qar Governorate. This result is consistent with a number of regional investigations carried out in Asian and Middle Eastern nations, where M. furfur has regularly been identified as the predominant species in superficial fungal infections.[19,20]

The climate of southern Iraq, which is marked by extended hot seasons and high humidity, may be linked to the high recovery rate of *M.furfur*. Lipid-dependent yeasts thrive in sebaceous-rich skin areas. Even though *M.globosa* was found in fewer samples, its occurrence indicates that several *Malassezia* species coexist in the local population. There have been reports of similar dispersion patterns in nearby nations,[21] implying that host-related, climatic, and environmental factors affect species predominance.

Several genetic groups within *M.furfur* were identified by the phylogenetic analysis, indicating significant intraspecies heterogeneity. When ribosomal and protein-coding gene targets are employed for molecular characterisation, this genetic heterogeneity has been extensively characterized.[22] Substitution mutations at particular nucleotide locations within the *β-tubulin* sequences were also found by multiple sequence alignment, suggesting continuous microevolutionary alterations among local isolates.

According to earlier molecular research, genetic diversity within *Malassezia* species may contribute to phenotypic variance, including possible variations in virulence and antifungal susceptibility.[23] The idea that regional strains might have distinct genetic signatures reflecting local environmental and host-related impacts is supported by the clustering pattern found in this investigation.

To the best of our knowledge, this study is the first molecular characterisation of *Malassezia* species in southern Iraq and the Thi-Qar Governorate. Local sequences deposited in the GenBank database offer important reference information for upcoming phylogenetic and epidemiological studies in Iraq and surrounding areas. When evaluating the results, some constraints should be taken into account, such as the uneven sample distribution and small control size. To further understand the molecular epidemiology of *Malassezia* species in Iraq, more extensive research using balanced sampling techniques and new genetic markers is advised.

Conclusions

Malassezia isolates from pityriasis versicolor patients in the Thi-Qar Governorate exhibit notable molecular diversity, according to the study. *Malassezia* isolates' pathogenic variability and adaptability may be impacted by this genetic diversity. These results highlight the necessity of molecular surveillance to direct efficient methods of diagnosis and treatment.

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Declaration of patient consent Authors certify that they have obtained all appropriate patient consent.

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Conflict of interest No conflict of interest.

Author's contribution

MAJ:. Substantial contribution to study design, manuscript writing and critical review of the manuscript

MHAY: Substantial contribution to data analysis and interpretation of data, manuscript writing.

Authors have given final approval of the manuscript version to be published and agreed to be accountable for all aspects of the work.

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