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Original Research Paper

Molecular Identification and Virulence Profiling of *Malassezia* spp. Associated with Inflammatory Dermatoses in Iraq

Raed A. H. Shabaa[1*], Rand M. A. Al-Husseini[2], Bassam M. Hameed[1], Ruqaya A. Hamza[1]

[1] *Department of Pathological Analyses, Faculty of Science, University of Kufa, Najaf, Iraq*

[2] *Department of Biology, Faculty of Science, University of Kufa, Najaf, Iraq*

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*Corresponding author: *Dr. Raed A. H. Shabaa Department of Pathological Analyses, Faculty of Science, University of Kufa, Najaf, Iraq*
Email: raaed.aboshibaa@uokufa.edu.iq

Abstract: *Malassezia* species are lipid-dependent yeasts that constitute part of the normal human skin microbiota but may contribute to inflammatory dermatoses under certain conditions. Integrated studies combining molecular identification with virulence-associated phenotypic traits remain limited in Iraq. **Objective:** This study aimed to characterize *Malassezia* isolates from inflammatory cutaneous lesions using combined phenotypic and molecular approaches. **Methods:** Seventy patients with clinically suspected *Malassezia*-associated dermatoses were examined between October 2024 and April 2025. Diagnostic evaluation included Wood's lamp examination, direct microscopy, and culture. Phenotypic characterization involved assessment of lipid dependence and virulence-associated traits, including catalase activity, biofilm formation, adherence to buccal epithelial cells, and extracellular enzyme activity. Molecular identification was performed by amplification and sequencing of the ITS1–5.8S–ITS2 region, with representative sequences deposited in GenBank (accession numbers PZ193669–PZ193671). **Results:** Culture growth was obtained in 82.9% of specimens. Three species were identified: *Malassezia globosa* (46.5%), *Malassezia furfur* (32.7%), and *Malassezia pachydermatis* (20.6%). Phylogenetic analysis of ITS sequences confirmed species-level identification, with isolates clustering into distinct clades corresponding to the identified species. Significant interspecies differences were observed in lipid dependence, biofilm formation, and extracellular enzymatic activity ($p < 0.001$), whereas catalase activity and epithelial adherence were consistently detected among isolates. **Conclusion:** These findings demonstrate species-specific variation in virulence-associated traits and highlight the value of integrating molecular identification, phylogenetic validation, and phenotypic profiling for improved understanding and diagnosis of *Malassezia*-associated dermatoses.

Keywords: *Malassezia*, ITS rDNA sequencing, biofilm formation, virulence factors, lipid dependence, inflammatory dermatoses

1. Introduction

Cutaneous fungal infections represent some of the most common infectious diseases worldwide and occur in a wide range of clinical presentations [1]. Among microorganisms inhabiting human skin, *Malassezia* species constitute lipid-dependent basidiomycetous yeasts that form an important component of the normal cutaneous mycobiome [2, 3]. Under physiological conditions, these organisms behave as commensals;

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however, alterations in skin microenvironment, immune status, or sebaceous activity may facilitate their transition toward opportunistic pathogenicity [2].

Species within the genus *Malassezia* consist of small, thick-walled yeast cells reproducing by unipolar budding and exhibiting marked lipid dependence, largely attributed to the absence of fatty acid synthase genes [4]. Colonization typically increases after puberty in association with enhanced sebaceous gland activity and modification of skin surface lipids [3, 4]. Commonly detected species include *M. globosa*, *M. restricta*, *M. furfur*, and *M. sympodialis*, with *M. globosa* and *M. restricta* frequently reported in adult populations and several dermatological disorders [3, 5].

Molecular epidemiological studies conducted worldwide consistently identify *M. globosa* and *M. restricta* as dominant species associated with pityriasis versicolor and seborrheic dermatitis [5, 6]. Similar distribution patterns have been reported across Middle Eastern regions. Investigations from Iran demonstrated predominance of *M. globosa* among isolates obtained from pityriasis versicolor patients [6], while studies conducted in Turkey confirmed frequent recovery of *M. globosa* and *M. restricta* from clinical samples [7]. In Iraq, previous work performed in An Najaf likewise reported *M. globosa* as the most frequently detected species in pityriasis versicolor cases [8]. Although regional investigations have described species distribution, comparatively limited attention has been directed toward virulence-associated characteristics of *Malassezia* species in inflammatory dermatoses.

Beyond superficial colonization, accumulating clinical and experimental evidence indicates involvement of *Malassezia* species in inflammatory conditions including seborrheic dermatitis and folliculitis [5, 9]. Pathogenic behaviour appears to depend not only on fungal presence but also on species-specific biological traits. Production of hydrolytic enzymes such as phospholipases, lipases, and proteases promotes survival within sebaceous environments and may contribute to disruption of the skin barrier and induction of inflammation [4]. Catalase activity further supports resistance to oxidative stress generated by host immune responses. In addition, biofilm formation represents an organized growth strategy capable of enhancing persistence and tolerance to antifungal exposure [9, 10]. Variability in these characteristics among isolates may therefore influence disease severity and treatment response.

Conventional diagnostic approaches, including microscopy and culture, remain essential in routine laboratory practice but offer limited resolution at the species level and cannot reliably distinguish colonization from active infection. Molecular identification based on amplification and sequencing of the internal transcribed spacer (ITS) region offers improved accuracy for epidemiological investigations [11, 12]. Accordingly, the present study aimed to characterize *Malassezia* isolates from inflammatory skin lesions in Iraqi patients by integrating ITS-based molecular identification with the assessment of species-specific virulence traits, thereby providing functional insight into their pathogenic potential and contribution to inflammatory dermatoses.

2. Methodology

Study design and patient population

This cross-sectional study was conducted at the Advanced Research Laboratory, Department of Pathological Analyses, Faculty of Science, University of Kufa, Najaf, Iraq, between October 2024 and April 2025. A total of 70 clinical specimens were collected from patients clinically suspected of *Malassezia*-associated dermatoses based on dermatological examination, characteristic lesion morphology, and Wood's lamp findings. Samples were obtained from patients attending Al-Sader Medical City, Najaf, Iraq, as well as several local dermatology clinics. Specimens were collected aseptically from affected cutaneous lesions for subsequent mycological and molecular analyses. The study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki. Informed consent was obtained from all participants prior to sample collection. The procedures involved non-invasive sampling and routine laboratory investigations, and no identifiable personal information was recorded.

Clinical examination and sample collection

Lesions were examined using a Wood's lamp (365 nm) in a darkened room. The lamp was positioned approximately 20 cm from the skin surface. Yellow-

white to copper-orange fluorescence was considered suggestive of *Malassezia* infection [13, 14]. Prior to sampling, affected areas were cleansed with 70% ethanol. Skin scrapings were obtained from the active lesion margins using a sterile blunt scalpel and transported in sterile folded black paper envelopes under dry conditions [15, 16]. Dandruff samples were collected using a sterile disposable comb [17].

Direct microscopic examination

Direct microscopic examination was performed using 10% potassium hydroxide (KOH) preparation to dissolve keratinized debris. The preparations were subsequently stained with lactophenol cotton blue to enhance visualization of fungal elements. Slides were examined under light microscopy for characteristic yeast and hyphal structures. The presence of clusters of thick-walled yeast cells with short hyphae producing the classical "spaghetti and meatballs" appearance was considered indicative of *Malassezia* spp. [16].

Culture and phenotypic characterization

Clinical specimens were inoculated onto Sabouraud dextrose agar (SDA) plates and incubated at 37 °C for a maximum of 7 days. To assess lipid requirement, selected SDA plates were supplemented by overlaying sterile olive oil (0.1 mL per plate). Plates were examined daily for visible growth [18, 19]. Colony appearance, including color, texture, margin characteristics, and surface consistency, was documented. Microscopic morphology was subsequently evaluated using lactophenol cotton blue staining to examine yeast cell shape, size, and budding pattern [20].

Molecular identification

Genomic DNA was extracted from isolate colonies using the Presto™ Mini gDNA Yeast Kit (Geneaid Biotech Ltd., Taiwan) according to the manufacturer's protocol. The internal transcribed spacer (ITS1–5.8S–ITS2) region of the ribosomal DNA was amplified using the universal primer pair ITS1 and ITS4 [21]. Polymerase chain reaction (PCR) was performed in a 20 µL final volume composed of 10 µL GoTaq® Green Master Mix (Promega, Madison, WI, USA), 1 µL of each forward and reverse primers (10 pmol μL^{-1}), 3 µL of DNA template, and 3 µL nuclease-free water. Thermal cycling conditions were initial denaturation at 95 °C for 5 minutes, followed by 30 cycles of denaturation at 95 °C for 30 seconds, annealing at 55 °C for 30 seconds, and extension at 72 °C for 45 seconds, then a final extension at 72 °C for 5 minutes. Amplified products were separated on 1.5% agarose gel prepared in TBE buffer, stained with nucleic acid dye, visualized under ultraviolet illumination, and documented using a gel documentation system [22]. Negative controls were included in all PCR reactions to exclude contamination. Representative ITS sequences were deposited in GenBank under accession numbers PZ193669, PZ193670 and PZ193671.

Phylogenetic analysis

The internal transcribed spacer (ITS) region sequences obtained in this study were aligned with reference sequences retrieved from GenBank using the MUSCLE algorithm implemented in MEGA version 12. Phylogenetic analysis was performed using the neighbor-joining (NJ) method based on the Kimura 2-parameter model. The robustness of the tree topology was evaluated using bootstrap analysis with 1000 replicates. Gaps and missing data were treated using pairwise deletion. A reference sequence of *Malassezia restricta* CBS 7877 (NR_103585) was included as an outgroup to root the tree.

Assessment of virulence factors

Catalase activity: Catalase activity was evaluated by adding one drop of 3% hydrogen peroxide to freshly grown colonies; immediate bubble formation was interpreted as a positive reaction [23].

Biofilm formation: Biofilm formation was assessed qualitatively using Congo red agar (CRA) containing brain heart infusion broth, sucrose, agar, and Congo red indicator. Plates were incubated at 37 °C for 24–48 hours. Black crystalline colonies were classified as strong biofilm producers, dark pink colonies as moderate producers, and red colonies as weak or non-biofilm producers [24].

Phospholipase production assay: Extracellular phospholipase activity was evaluated using an egg yolk

agar plate method. The medium consisted of Sabouraud dextrose agar supplemented with sterile egg yolk emulsion (10%). Fresh yeast colonies were spot-inoculated onto the surface of the medium and incubated at 37 °C for 48–72 h. Following incubation, plates were examined for the presence of a visible precipitation zone surrounding the colony, indicative of phospholipid hydrolysis. Isolates showing a distinct precipitation zone were classified as phospholipase-positive, whereas isolates without a visible zone were considered phospholipase-negative. This method is widely used for the qualitative detection of extracellular phospholipase activity in yeasts [25, 26] and reflects their ability to hydrolyse host membrane lipids, which may contribute to pathogenicity in *Malassezia* species [4].

Lipase production assay: Extracellular lipase activity was evaluated using Rhan medium containing a lipid substrate (Tween 80) as the primary carbon source. Fresh yeast colonies were spot-inoculated onto the surface of the medium and incubated at 37 °C for 48–72 h. Following incubation, plates were examined for the presence of an opaque precipitation zone surrounding the colonies, resulting from calcium soap formation due to lipid hydrolysis. Isolates showing a visible precipitation zone were classified as lipase-positive, whereas those without a detectable zone were considered lipase-negative. This assay provides a qualitative indication of extracellular lipase activity and reflects the ability of *Malassezia* species to utilize host-derived lipids [4].

Adherence to buccal epithelial cells: Adherence to buccal epithelial cells (BECs) was evaluated by incubating standardized yeast suspensions with freshly collected BECs at 37 °C for one hour. After centrifugation and washing to remove non-adherent cells, smears were prepared, fixed, stained with crystal violet, and examined microscopically to determine adherence capacity [24].

Statistical analysis

Statistical analysis was performed using SPSS software version 26 (IBM Corp., USA). Descriptive statistics were expressed as frequencies and percentages. Associations between clinical source, species distribution, and virulence factors were analysed using the Chi-square test. A p-value < 0.05 was considered statistically significant.

3. Results and discussion

Clinical findings

Seventy patients presenting with clinically suspected *Malassezia*-associated dermatoses were included in this study. Clinical presentation was heterogeneous and comprised pityriasis versicolor in both hypopigmented and hyperpigmented forms, erythematous inflammatory lesions, and seborrheic dermatitis. Representative clinical lesions observed under normal illumination are presented in Figure 1 (A–D). These images show the typical pigmentary changes seen in pityriasis versicolor, including both hypopigmented and hyperpigmented patches, as well as inflammatory plaques located predominantly in sebaceous-rich anatomical areas.

Wood's lamp examination served as a supportive diagnostic tool in cases suggestive of pityriasis versicolor. When examined under ultraviolet light (365 nm), affected areas displayed a characteristic yellow-white to copper-orange fluorescence. The fluorescence closely corresponded to clinically involved skin and allowed clearer visualization of lesion boundaries compared with standard illumination. Examples of these fluorescence patterns are shown in Figure 1 (E–H).

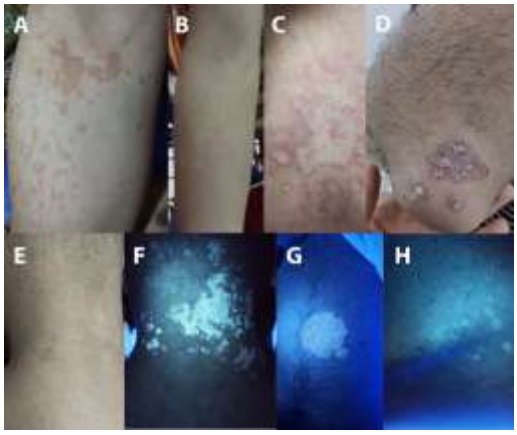

Fig 1: Clinical features of Malassezia-associated dermatoses. (A–E) Lesions observed under normal light: (A, E) hyperpigmented pityriasis versicolor; (B) hypopigmented pityriasis versicolor; (C) erythematous scaly plaque; and (D) seborrheic dermatitis involving the scalp margin. (F–H) Corresponding Wood's lamp

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examination (365 nm) demonstrating characteristic yellow–white to copper–orange fluorescence.

Direct microscopic examination

Direct microscopic evaluation of skin scrapings treated with 10% potassium hydroxide and lactophenol cotton blue revealed numerous thick-walled spherical yeast cells accompanied by short hyphal elements, forming the classical "spaghetti and meatballs" morphology (Figure 2). These findings confirmed active fungal proliferation in the majority of clinically suspected cases.

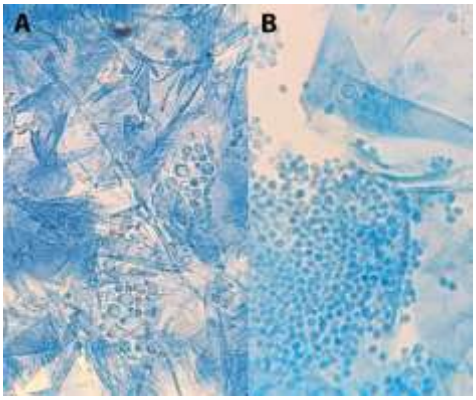

Fig 2: Direct microscopic examination of clinical skin scrapings. Skin scrapings were examined using potassium hydroxide (KOH) preparation and lactophenol cotton blue staining (×1000). (A) Presence of yeast cells with short hyphal elements ("spaghetti and meatballs" appearance). (B) Numerous spherical budding yeast cells clustered near epithelial fragments.

Culture characteristics

Culture yielded growth in 58 of 70 specimens (82.9%). Colonies grown on Sabouraud dextrose agar appeared creamy to pale yellow, smooth to slightly lobulated, convex, and exhibited a characteristic buttery consistency with well-defined margins (Figure 3A).

Microscopic examination of cultured isolates stained with lactophenol cotton blue demonstrated oval to ellipsoidal budding yeast cells with distinct collarette formation at the budding site (Figure 3B), consistent with morphological characteristics of the genus *Malassezia*.

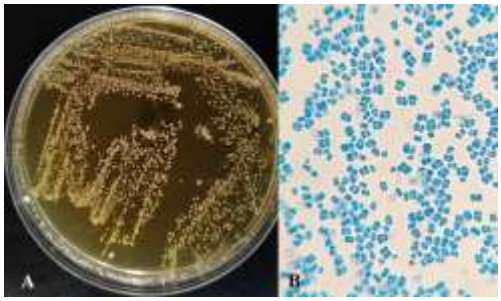

Fig 3: Morphological characteristics of Malassezia isolates. (A) Macroscopic colony morphology on Sabouraud dextrose agar showing creamy to pale-yellow, smooth, convex colonies with well-defined margins. (B) Microscopic morphology stained with lactophenol cotton blue (×1000), revealing oval to ellipsoidal budding yeast cells.

Molecular identification

The amplification of the ITS1–5.8S–ITS2 regions was obtained for all 58 isolates included in this study. Agarose gel electrophoresis revealed clear bands corresponding to the expected amplicon sizes for the ITS region (Figure 4).

The PCR products were subsequently sequenced by the Sanger method at Macrogen, Korea. The sequences were compared with RefSeq entries in the NCBI GenBank database using the BLAST search. Sequence similarity values ranged between 98% and 100% when aligned with reference strains.

Twenty-seven isolates (46.5%) exhibited 98–100% similarity to *Malassezia globosa* strain ATCC MYA-4612 (NR_111475.1), corresponding to an ITS amplicon size of approximately 674 bp. Nineteen isolates (32.7%) showed 98–100% similarity to *M. furfur* strain CBS 7019 (NR_149347.1), corresponding to an ITS amplicon size of approximately 789 bp. The remaining twelve isolates (20.6%) aligned with *M. pachydermatis* strain CBS 1879 (NR_126114.1), corresponding to an ITS amplicon size of approximately 755 bp and sequence similarity values ranging from 99% to 100%. Species assignment was based on the highest BLAST similarity in conjunction with concordant phenotypic characteristics. Representative electrophoretic profiles are shown in Figure 4.

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Phylogenetic analysis of ITS sequences revealed clear clustering of the isolates into distinct species-level groups (Figure 5). The isolates identified as *Malassezia furfur*, *Malassezia pachydermatis*, and *Malassezia globosa* formed well-supported clades with their respective reference sequences.

The *M. furfur* cluster showed strong bootstrap support (84–100%), while *M. pachydermatis* and *M. globosa* isolates also grouped with high confidence (bootstrap values up to 100%). The outgroup *M. restricta* CBS 7877 was clearly separated from the studied isolates, confirming the phylogenetic structure of the tree.

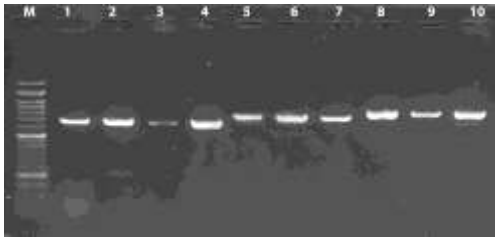

Fig 4: Agarose gel electrophoresis of ITS amplicons. Amplification of ITS1–5.8S–ITS2 regions of Malassezia species. Lane M: DNA size marker. Lanes 1–4: Malassezia globosa (~674 bp); lanes 5–7: M. pachydermatis (~755 bp); lanes 8–10: M. furfur (~789 bp).

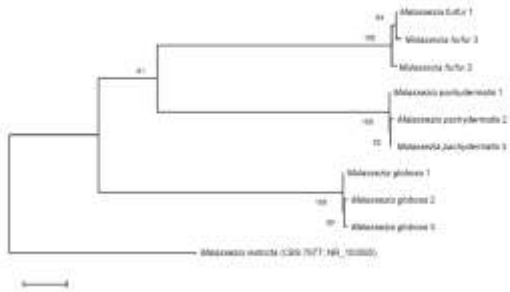

Fig 5: Phylogenetic tree based on ITS region sequences of *Malassezia* isolates. Evolutionary distances are expressed as the number of nucleotide substitutions per site. *Malassezia restricta* CBS 7877 (NR_103585) was used as an outgroup.

Assessment of virulence factors

Marked differences in virulence-associated phenotypic traits were observed among *Malassezia* species isolates. Lipid dependence demonstrated a clear species-specific pattern. All *M. pachydermatis* isolates (12/12) exhibited growth on Sabouraud dextrose agar both with and without olive oil supplementation, indicating relative lipid independence, whereas *M. globosa* (27/27) and *M. furfur* (19/19) isolates required lipid supplementation for growth. This association between species identity and lipid requirement was statistically significant ($p < 0.001$).

Biofilm formation ability differed significantly among species ($p < 0.001$) as shown in Table 1. The majority of *M. pachydermatis* isolates (10/12; 83.3%) were classified as strong biofilm producers, whereas *M. furfur* demonstrated intermediate capacity, with 11 of 19 isolates (57.9%) identified as strong producers. In contrast, only 6 of 27 *M. globosa* isolates (22.2%) exhibited strong biofilm formation (Figure 6).

Table 1: Biofilm formation capacity of *Malassezia* species

Species	**No. of isolates**	**Strong n (%)**	**Moderate n (%)**	**Weak or Non n (%)**
Malassezia globose	27	6 (22.2)	12 (44.4)	9 (33.3)
Malassezia furfur	19	11 (57.9)	5 (26.3)	3 (15.8)
Malassezia pachydermatis	12	10 (83.3)	2 (16.7)	0 (0.0)

Chi-square test: $p < 0.001$

*Statistically significant ($p < 0.05$).

Fig 6: Congo red agar (CRA) assay for biofilm formation. Representative phenotypes of Malassezia isolates on Congo Red Agar (CRA) medium. Strong biofilm producers (S) exhibit black, dry crystalline colonies; moderate producers (M) show dark pink colonies; weak or non-biofilm producers (W) display red colonies.

Catalase activity was detected in all isolates tested, indicating a consistent capacity for oxidative stress resistance among *Malassezia* species under the experimental conditions. Adherence to buccal epithelial cells was observed in all isolates. Microscopic

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examination confirmed the presence of yeast cells attached to the epithelial surface in all samples examined (Figure 7), indicating a uniform capacity for epithelial adherence among the tested isolates.

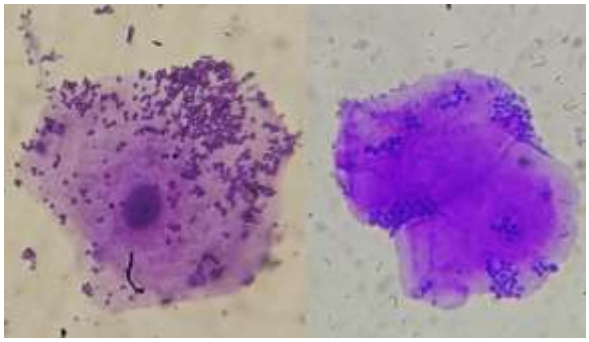

Fig 7: Adherence of Malassezia isolates to human buccal epithelial cells. Light microscopy (×1000) of epithelial cells stained with crystal violet showing adherence of yeast cells. Malassezia cells appear as clustered round to oval elements attached to the epithelial surface.

Extracellular enzyme activity also varied significantly among species ($p < 0.001$) (Table 2). Phospholipase activity was detected in all *M. pachydermatis* isolates (12/12; 100%), whereas it was absent in *M. globosa* (0/27; 0.0%) and observed in only a small proportion of *M. furfur* isolates (3/19; 15.8%) (Figure 8A). In contrast, lipase activity was highly prevalent in *M. globosa* (26/27; 96.3%) and *M. pachydermatis* (11/12; 91.7%), but was less frequently detected in *M. furfur* (8/19; 42.1%) (Figure 8B). Overall, lipase activity was more commonly observed than phospholipase activity among the isolates. These findings indicate distinct species-specific patterns in extracellular enzymatic activity.

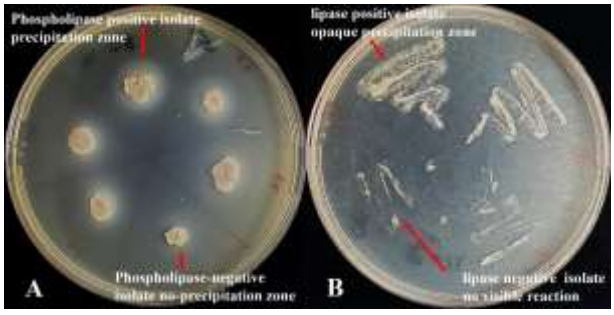

Fig 8: Enzymatic activity of Malassezia isolates. (A) Phospholipase activity indicated by a clear precipitation halo surrounding colonies (positive isolates), while its absence indicates negative activity. (B) Lipase activity indicated by opaque precipitation zones around streaked growth (positive isolates), whereas lipase-negative isolates show no visible reaction.

Table 2: Extracellular enzyme activity among *Malassezia* species

Species	**No. of isolates**	**Phospholipase positive n (%)**	**Phospholipase negative n (%)**	**Lipase positive n (%)**	**Lipase negative n (%)**
Malassezia globose	27	0 (0.0)	27 (100)	26 (96.3)	1 (3.7)
Malassezia furfur	19	3 (15.8)	16 (84.2)	8 (42.1)	11 (57.9)
Malassezia pachydermatis	12	12 (100)	0 (0.0)	11 (91.7)	1 (8.3)
Total	58	15 (25.9)	43 (74.1)	45 (77.6)	13 (22.4)

Chi-square test: Phospholipase: $p < 0.001$; Lipase: $p < 0.001$

*Statistically significant ($p < 0.05$).

This study provides a comprehensive clinical, phenotypic, and molecular characterization of *Malassezia*-associated dermatoses in Najaf, Iraq. By integrating conventional diagnostic approaches with ITS-based molecular identification, phylogenetic analysis, and evaluation of virulence-associated traits, the present work offers a more robust understanding of species distribution and pathogenic variability within this genus.

Consistent with previous reports, *M. globosa* was the most frequently identified species, followed by *M. furfur* and *M. pachydermatis*. Globally, *M. globosa* and *M. restricta* are commonly associated with pityriasis versicolor and seborrheic dermatitis [9, 30]. Similar distribution patterns have been reported in regional studies from Iran and Turkey, where *M. globosa* predominance has been consistently observed [17, 31]. Variations in species prevalence across different geographical regions have been attributed to environmental factors, host characteristics, and methodological differences [29, 31]. The findings of the present study are therefore consistent with both global and regional epidemiological trends, while contributing novel data from Iraq.

Importantly, phylogenetic analysis based on ITS sequences provided additional validation of species-

level identification and strengthened the reliability of molecular findings. The constructed phylogenetic tree demonstrated clear clustering of the isolates into distinct, well-supported clades corresponding to *M. furfur*, *M. pachydermatis*, and *M. globosa*. High bootstrap values across major nodes indicate strong confidence in the inferred relationships. The use of *M. restricta* CBS 7877 as an outgroup enabled proper rooting of the tree and confirmed the evolutionary separation between the reference lineage and the studied isolates. These results support the accuracy of ITS-based identification and are consistent with previous studies demonstrating the utility of ITS regions for resolving *Malassezia* species. Nevertheless, it is important to recognize that ITS sequences may have limited discriminatory power among closely related taxa, and future investigations employing multi-locus sequence analysis (MLSA) could provide improved resolution and deeper insight into genetic diversity.

In addition to molecular identification, the present study highlights the importance of phenotypic characteristics associated with pathogenicity. Lipid dependence exhibited clear species-specific variation, with *M. globosa* and *M. furfur* demonstrating strict lipid requirements, whereas *M. pachydermatis* showed relative lipid independence. This observation reflects fundamental metabolic differences within the genus and is consistent with previous findings [23].

A key finding of this study is the marked interspecies variation in biofilm-forming capacity. *M. pachydermatis* exhibited the highest proportion of strong biofilm producers, whereas *M. globosa* demonstrated comparatively limited biofilm formation. Biofilm formation represents an important virulence strategy that enhances microbial persistence, structural organization, and resistance to antifungal agents [3, 4, 28, 29]. The increased tolerance of biofilm-associated cells to antifungal therapy further highlights its clinical significance. Antifungal resistance among fungal pathogens has been increasingly reported in clinical settings, posing a significant challenge to effective treatment strategies [28, 32]. These findings suggest that species-specific differences in biofilm formation may influence disease persistence and therapeutic outcomes.

The observed variation in biofilm formation may be partially explained by ecological adaptation. *M. globosa* is primarily associated with human skin and is well adapted to lipid-rich environments, where it commonly exists as a commensal organism [30, 13]. This stable host adaptation may reduce the necessity for strong biofilm formation. In contrast, *M. pachydermatis*, which is commonly associated with animal hosts, demonstrates broader ecological flexibility and lower lipid dependence [23], potentially relying more on biofilm formation as a survival mechanism in variable environments.

Extracellular enzymatic activity also demonstrated pronounced interspecies differences. Phospholipase activity was detected in all *M. pachydermatis* isolates and in a minority of *M. furfur* isolates, while it was absent in *M. globosa*. In contrast, lipase activity was highly prevalent in *M. globosa* and *M. pachydermatis*, but less frequent in *M. furfur*. These findings reflect differences in lipid metabolism and host interaction. Lipases play a crucial role in the utilization of sebum-derived lipids, facilitating colonization and growth on human skin, whereas phospholipases contribute to disruption of host cell membranes and may enhance inflammatory responses [13]. Such enzymatic variability may therefore influence the pathogenic potential and clinical manifestations associated with different *Malassezia* species.

Catalase activity was uniformly detected in all isolates, indicating a conserved mechanism for protection against oxidative stress. By neutralizing reactive oxygen species generated during host immune responses, catalase contributes to fungal survival within inflamed skin environments [13, 27]. Although this trait did not differ between species, its universal presence suggests an essential role in *Malassezia* persistence.

All isolates demonstrated the ability to adhere to buccal epithelial cells, confirming their capacity to attach to host epithelial surfaces. Adhesion is a critical step in colonization and enables sustained interaction with host tissues [3, 4]. Although no interspecies differences were observed, the consistent adherence capacity supports the role of *Malassezia* species as effective colonizers of epithelial environments.

Overall, the findings of this study indicate that the pathogenic potential of *Malassezia* species is multifactorial and species-dependent. The integration of

phylogenetic analysis with molecular identification and phenotypic characterization provides a more comprehensive framework for understanding species diversity, evolutionary relationships, and pathogenic mechanisms. Variations in lipid utilization, biofilm formation, extracellular enzymatic activity, and oxidative stress resistance collectively contribute to differences in colonization, persistence, and host interaction. These insights may contribute to improved diagnostic strategies and targeted therapeutic approaches in *Malassezia*-associated dermatoses.

Conclusion

The present study combined ITS-based molecular identification, phylogenetic analysis, and phenotypic characterization to investigate *Malassezia* species associated with inflammatory dermatoses in Najaf, Iraq. Phylogenetic analysis confirmed species-level identification and demonstrated clear clustering of isolates into well-supported clades, strengthening the reliability of molecular findings. Significant interspecies differences were identified in lipid dependence, biofilm formation, and extracellular enzymatic activity, while catalase activity and epithelial adherence were consistently observed among isolates.

These findings highlight the role of species-specific virulence-associated traits, particularly biofilm formation, lipid utilization, and oxidative stress resistance, in influencing persistence and host interaction. The integration of molecular identification, phylogenetic validation, and phenotypic profiling provides a more comprehensive understanding of *Malassezia* biology and may contribute to improved diagnostic approaches and targeted therapeutic strategies for *Malassezia*-associated skin infections.

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Author contributions

Raed A. H. Shabaa supervised the study and contributed to manuscript preparation. Rand M. A. Al-Husseini performed the molecular experiments and analysed and interpreted the data. Bassam M. Hameed performed specimen collection and assisted in laboratory work. Ruqaya A. Hamza carried out laboratory experiments and data collection. All authors read and approved the final manuscript.

Ethics

This study was conducted under approval by the medical ethics committee at the University of Kufa (2017). Verbal and written consent was provided by parents and agreement for publication was obtained from both participants and researchers

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