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Scraping enhances microbial DNA recovery over swabbing in sensitive facial skin: a pilot study of 10 patients

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Sensitive skin is a common condition with a complex pathogenesis involving both host-related factors and microbial interactions. Emerging evidence suggests a bidirectional relationship between skin microbiome dysbiosis and sensitive skin, although whether microbial shifts are causal or consequential remains unclear. Characterizing the microbiome in this population is particularly challenging due to low microbial biomass and heightened skin reactivity, both of which may compromise sampling and data quality. While swabbing remains the most common method for skin microbiome collection, it may fail to yield sufficient DNA, especially from delicate facial areas. In this pilot study of 10 patients with sensitive facial skin, we compared swabbing to gentle scraping using a sterile No. 10 surgical blade. Swabbing consistently failed to recover detectable microbial DNA. In contrast, scraping was well-tolerated and enabled one-time sampling that yielded sufficient DNA for both bacterial and fungal sequencing. DNA concentrations ranged from 0.065 to 13.2 ng/μL for bacteria and 0.104 to 30.0 ng/μL for fungi. Genus-level classification rates exceeded 99.7% for bacteria and 97% for fungi. Shannon diversity indices ranged from 0.03 to 2.85 for bacteria and 0.106 to 2.849 for fungi. PCoA revealed substantial inter-individual variation in community composition. Dominant taxa included *Staphylococcus aureus* group, *Cutibacterium acnes* group, *Malassezia restricta*, and *Malassezia globosa*. These findings indicate that skin scraping is a feasible and reproducible method for microbiome studies of sensitive skin, providing comprehensive taxonomic and ecological profiling in a single, non-invasive session.

Sensitive skin is a common dermatologic condition characterized by subjective symptoms of stinging, burning, or itching in response to stimuli that typically should not provoke such reactions[1]. Epidemiological studies report a prevalence of 50–60% in women and up to 40% in men globally[2]. Its pathogenesis is multifactorial, involving impaired barrier function, increased nerve sensitivity, and dysregulated immune responses[3,4].

In recent years, attention has turned to the potential role of the skin microbiome in the etiology and maintenance of sensitive skin[5]. The skin microbiome, comprising bacteria, fungi, and viruses, plays key roles in immune modulation, barrier defense, and inflammation control[6,7]. Disruptions in this microbial ecosystem have been linked to skin disorders such as atopic dermatitis, acne, and rosacea[8–10]. Although less well-studied, evidence is emerging that sensitive skin may also be associated with altered microbiome composition, including decreased *Staphylococcus epidermidis* and increased *Cutibacterium acnes* imbalance[11,12].

Sampling the skin microbiome from sensitive facial areas presents specific methodological and biological challenges. The low microbial biomass in certain facial regions, compounded by frequent cleansing or cosmetic use, can hinder adequate DNA recovery[13]. Moreover, sampling techniques must avoid exacerbating symptoms in patients whose skin already reacts to mild friction or touch[14]. Most previous studies have used swabbing a 2 cm

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× 2 cm patch of skin for microbial analysis[15], but some investigators report difficulty recovering DNA suitable for sequencing, especially in sensitive or dry skin types[16].

Alternative methods include tape stripping, cup scrubbing, or skin biopsies. However, these may cause discomfort, require special equipment, or pose ethical concerns[17,18]. In a recent study, Ogai et al. compared tape stripping with swabbing and found significantly higher DNA recovery with tape, but also noted increased skin irritation[19]. Therefore, a balance between efficacy and safety is needed in choosing sampling methods for sensitive skin.

This study was initiated following our real-world experience in which standard swabbing failed to yield measurable microbial DNA in sensitive-skin patients. We developed a gentle scraping technique using a sterile surgical blade to recover stratum corneum samples with minimal discomfort. We hypothesized that this method would provide higher DNA yields, support successful bacterial and fungal sequencing, and enable one-time sampling, improving patient experience and data quality.

Materials and methods
Study design and ethics approval
This was a pilot study involving 10 adult patients diagnosed with facial sensitive skin. Patients were recruited from the Department of Dermatology and Skin Aesthetics, University Medical Center, Ho Chi Minh City. All participants provided informed written consent. The study was approved by the Institutional Review Board of the University of Medicine and Pharmacy at Ho Chi Minh City (*382/HĐĐĐ-ĐHYD*, *22/03/2023*), in accordance with the Declaration of Helsinki.

Patient selection criteria
Participants aged 18 to 55 years, of any gender, were enrolled in the study if they were clinically diagnosed with sensitive facial skin by a board-certified dermatologist, and got a positive score on a validated sensitive skin questionnaire[1] and lactic acid sting test. Exclusion criteria included the following: (1) presence of any active or chronic dermatologic condition affecting the face; (2) use of systemic or topical antibiotics, antifungal agents, corticosteroids, oral isotretinoin, probiotics or topical antiseptics within two weeks prior to sample collection; (3) current diagnosis of any serious systemic illness, including but not limited to cardiovascular, hepatic, renal disease, severe autoimmune disorders, or active malignancy; (4) receipt of dermatological procedures involving the face within the past three months, such as chemical peeling, laser treatment, intense pulsed light (IPL), radiofrequency (RF), high-intensity focused ultrasound (HIFU), or mesotherapy; (5) pregnancy or breastfeeding.

Sampling protocol
Participants were instructed to avoid applying any topical products to the face, including moisturizers, cosmetics, and sunscreens, for at least 24 h prior to sampling. In addition, they were advised to wash their face with plain water only (without using facial cleansers or soaps) during this period to minimize potential interference with the skin's microbiome composition.

All skin microbiome samples were collected in a dedicated sampling room maintained at a stable temperature of 22 ± 2 °C and relative humidity of $50\% \pm 10\%$. The sampling process was performed under sterile conditions to prevent environmental contamination. Participants were given detailed instructions on skin care regimens for 1 week after the first visit to ensure eligibility for the selection criteria of this study if this first sampling attempt was unsuccessful.

Initial swabbing attempt
At the first visit, samples were collected using sterile cotton swab (Ningbo Greetmed Medical Instruments Co, Ltd). Skin swab samples were collected from the entire facial surface, including the forehead, temples, nose, chin, medial and lateral cheeks, and jawline. Each swab was moistened with sterile phosphate-buffered saline (PBS) prior to contact with the skin. The operator wore sterile gloves and used one hand to gently stretch and stabilize the patient's facial skin, while the other hand performed the swabbing.

Swabbing was conducted using a moderate and consistent pressure, applying circular motions while simultaneously rotating the swab to ensure that all sides of the cotton tip made contact with the skin. Approximately 20 circular strokes were applied per subregion before moving to the adjacent area. A total of 8 swabs were used to comprehensively sample the entire face.

The collected swabs were immediately cut and the cotton heads were placed into two sterile 15 mL Falcon tubes, each pre-filled with 3 mL of PBS for DNA preservation. One tube was designated for bacterial analysis and the other for fungal analysis, with 4 swabs in each tube. Tubes were securely capped and manually shaken for 30 seconds to ensure uniform dispersion of cellular material.

All samples were immediately sealed with parafilm and transported on ice to the molecular biomedicine center, where they were stored at 4 °C for no longer than 3 days before DNA extraction was performed. The extracted DNA samples were subsequently stored at - 80 °C.

Skin scraping protocol
Due to poor DNA yield with swabbing, patients were recalled for a second visit. Skin scraping was performed in the same sampling room as the swabbing procedure, under identical environmental conditions (temperature and humidity), and with strict adherence to sterile technique throughout the entire process to minimize the risk of external contamination.

For scraping microbiome sampling, one hand was used to gently stretch and stabilize the skin above the target area, ensuring the skin remained taut during the procedure. The other hand held a No. 10 sterile surgical

blade (Doctor™, India) using the thumb and index finger, while the fifth finger rested lightly on the patient's skin surface to provide support and control.

The blade was positioned at a 15 - 30° angle relative to the skin, and only the curved portion of the blade, not the pointed tip, was allowed to contact the surface, minimizing the risk of abrasion or injury. Using light, controlled pressure, the operator gently scraped along the skin surface in a downward motion. Each linear stroke was repeated approximately 10 times before proceeding to the adjacent area, continuing until the entire region was covered.

Superficial stratum corneum fragments adhering to the blade were immediately transferred onto a pre-moistened sterile cotton swab (moistened with sterile PBS). Once sufficient material was collected on the swab, the cotton head was cut and placed into a sterile 15 mL Falcon tube containing 3 mL of PBS to preserve microbial DNA. Each tube contained four swabs and was assigned either for bacterial or fungal DNA analysis. A total of eight swabs were used to sample the entire face.

The tubes were tightly sealed and manually agitated for 30 seconds to disperse the collected material. Sample preservation and transport protocols were identical to those used for swab-collected samples. All samples were immediately sealed with parafilm and transported on ice to the molecular biomedicine center, where they were stored at 4 °C for no longer than 3 days before DNA extraction was performed. The extracted DNA samples were subsequently stored at - 80 °C.

DNA extraction and quantification

DNA extraction and quantification - bacterial DNA
Both skin sampling methods, swabbing and scraping, underwent the same standardized bacterial DNA extraction protocol. For each sample, 1 mL of Depletion Solution was added directly to a 15 mL Falcon tube containing 4 PBS-moistened sterile cotton swabs (previously used to collect skin material in 3 mL PBS). The tubes were rotated end-over-end for 15 minutes at room temperature (20 - 30 °C), followed by brief vortexing (10 - 15 seconds) and centrifugation for 2 minutes to pellet the sample.

The pellet was carefully transferred into a 1.5 mL microcentrifuge tube, then centrifuged at $10,000 \times g$ for 5 minutes. The supernatant was removed without disturbing the pellet, leaving a maximum volume of 200 µL. DNA extraction was then continued using the HostZERO™ Microbial DNA Kit (Zymo Research, USA), following steps 5 through 10 of the Host DNA Depletion and Microbial DNA Isolation protocols provided by the manufacturer.

In step 5 of the Microbial DNA Isolation workflow, the filtration process was repeated 2 - 3 times as needed to ensure complete processing of the sample. In step 10, the incubation time was extended to 10 minutes, and 20 µL of filtrate was applied to the column, incubated for an additional 30 minutes, and then centrifuged at full speed for 1 minute to elute the DNA.

Final DNA concentrations were quantified using the Qubit™ 4 Fluorometer with the High Sensitivity dsDNA Assay Kit (Thermo Fisher Scientific). According to the manufacturer's specifications, this assay has a lower detection limit of 0.0005 ng/µL. Samples with DNA concentrations below this threshold were automatically reported as "Sample out of range TOO LOW", indicating that the DNA quantity was insufficient for reliable quantification and downstream analysis.

DNA extraction and quantification - fungal DNA
Both skin sampling methods, swabbing and scraping, underwent the same standardized fungal DNA extraction protocol. Each 15 mL Falcon tube containing 4 PBS-moistened sterile cotton swabs was vortexed for 10–15 seconds to release skin flakes into the solution. The tubes were then centrifuged for 3.5 minutes to sediment skin material.

The pellet was transferred to a 1.5 mL microcentrifuge tube and centrifuged at maximum speed ($15,000 \times g$) for 15 minutes. Without disturbing the pellet, the supernatant was carefully removed, leaving a final volume of approximately 200 µL. The 200 µL sample was transferred to a ZR BashingBead™ Lysis Tube (0.1 mm & 0.5 mm), followed by the addition of 750 µL of BashingBead™ Buffer.

The sample was processed at maximum speed in a bead beater for 10 minutes. The tube was then centrifuged at $10,000 \times g$ for 1 minute, and up to 600 µL of the supernatant was transferred to a Zymo-Spin™ III-F Filter in a Collection Tube and centrifuged at $8,000 \times g$ for 1 minute.

Subsequent steps followed the manufacturer's protocol (steps 5 - 10) from the Quick-DNA™ Fungal/Bacterial Miniprep Kit (Zymo Research, USA). In step 10, 40 µL of DNA Elution Buffer was added directly to the column matrix and incubated for 20 minutes. The column was then centrifuged at $10,000 \times g$ for 1 minute. The eluted volume was reapplied to the same column, incubated again for 20 minutes, and centrifuged a second time to maximize DNA recovery.

Fungal DNA concentrations were measured using the Qubit™ 4 Fluorometer with the High Sensitivity dsDNA Assay Kit (Thermo Fisher Scientific). This assay has a lower detection limit of 0.0005 ng/µL, as specified by the manufacturer. Samples with DNA concentrations below this threshold were recorded as "Sample out of range TOO LOW", indicating that the fungal DNA content was insufficient for reliable quantification and could not be used for sequencing.

Library preparation and sequencing

Bacterial libraries were prepared using the Quick-16 S Plus NGS Library Prep Kit (D6421, Zymo Research), targeting the V3 - V4 region of the 16 S rRNA gene. Fungal DNA libraries were prepared using the Quick-ITS Plus NGS Library Prep Kit (D6425) for ITS1 sequencing. All libraries were sequenced using the Illumina BaseSpace™ platform. Raw sequence files were processed through the BaseSpace 16 S Metagenomics and ITS pipelines, which included quality filtering, taxonomic classification, and abundance estimation.

Data analysis

For bacterial analysis, 16 S rRNA sequencing data were processed using the EzBioCloud platform (www.ezbiocloud.net). Following quality filtering, chimera removal, and sequence alignment, taxonomic classification was performed using the EzBioCloud 16 S rRNA reference database. Taxonomic profiles were analyzed down to the species level. Diversity metrics, including Shannon index and operational taxonomic unit (OTU) counts, were calculated for each sample and exported for downstream analysis. Taxonomic composition at the phylum, genus, and species levels was visualized using stacked bar plots. Genera and species representing less than 1% relative abundance were grouped under "ETC" (others).

For fungal analysis, ITS1 sequencing data were processed on the Illumina BaseSpace™ platform. Taxonomic classification was assigned using the UNITE reference database. Relative abundance bar plots were generated at the genus and species levels for fungal taxa.

Statistical analyses were performed using Python (version 3.11) with the SciPy library for non-parametric testing, and Seaborn and Matplotlib for data visualization. Wilcoxon signed-rank tests were applied to compare microbial DNA yields between swabbing and scraping, and between bacterial and fungal DNA concentrations within the scraping method. Spearman's rank correlation was used to assess associations between DNA concentration and Shannon diversity indices, as well as between bacterial and fungal alpha diversity. A significance threshold of $p < 0.05$ was used for all statistical tests.

Results

Swabbing failed to recover microbial DNA

At the first visit, all swab samples showed DNA concentrations below the detection threshold (< 0.0005 ng/μL), as confirmed by Qubit™ 4 Fluorometer (shown in Supplementary Fig. 1). No measurable bacterial or fungal DNA was recovered, preventing downstream sequencing. This outcome necessitated a second sampling using an alternative method.

Scraping enabled successful DNA extraction

Scraping performed during the second visit yielded measurable microbial DNA in all 10 patients. Bacterial DNA concentrations had a median of 0.109 ng/μL (interquartile range [IQR]: 0.1035 - 0.1965), while fungal DNA concentrations showed a higher median of 0.9525 ng/μL (IQR: 0.1840 - 3.9000). These results suggetsts that scraping may provide a more effective approach than swabbing for recovering sufficient DNA for metagenomic analysis from sensitive facial skin. (Figures 1 and 2, Supplementary Fig. 2–4).

Comparative yield analysis

Comparative statistical analysis confirmed that scraping yielded significantly higher DNA concentrations than swabbing for both bacteria and fungi ($p = 0.00195$ for both comparisons, Wilcoxon signed-rank test). While all swab samples remained at the lower detection limit (< 0.0005 ng/μL), scraping allowed recovery of quantifiable DNA in all patients in this pilot study. These differences were also clearly visualized in boxplots comparing DNA yield by method and microbial type (Fig. 3).

Scraping yielded significantly higher DNA levels than swabbing ($p < 0.01$ for both comparisons).

Within the scraping method, fungal DNA concentrations were significantly higher than bacterial DNA concentrations across all patients ($p = 0.00195$, Wilcoxon signed-rank test). This difference suggests that fungal biomass may be more abundant or more readily recovered from the stratum corneum in sensitive facial skin (Fig. 4).

Bacterial sequencing and community structure

All 10 scraped samples yielded sufficient bacterial DNA for sequencing, with genus-level classification rates above 99.7%. Shannon diversity had a median of 0.30 (IQR: 0.13 - 0.65), while OTUs ranged from 6 to 325 with a median of 15.5 (IQR: 8.75 - 41.25), confirming substantial inter-individual variability even in standardized sampling. VK-M13 exhibited the highest bacterial diversity, while VK-M9 and VK-M11 had the lowest (Table 1).

Across taxonomic levels, *Firmicutes*, *Proteobacteria*, and *Actinobacteria* were dominant phyla. *Staphylococcus*, *Cutibacterium*, and *Pluralibacter* were key genera, while species like *C. acnes* and *S. aureus* were commonly detected. These profiles support the scraping method as a useful approach to recover diverse bacterial communities from facial skin (Fig. 5).

**Fig. 1**. Scraping performed with No. 10 surgical blade showing gentle technique with no skin injury.
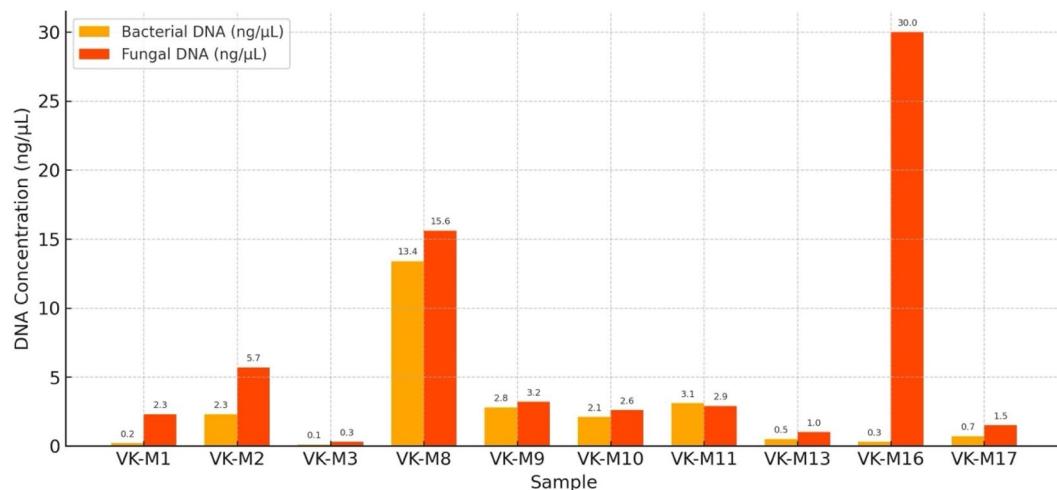
**Fig. 2**. Barplot comparing bacterial and fungal DNA yields per patient.
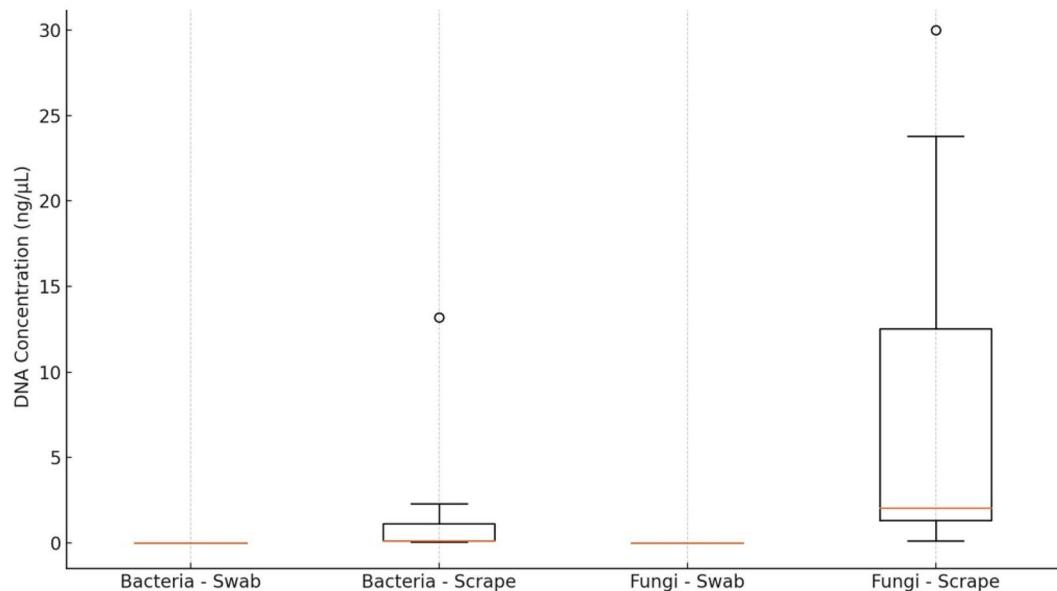
Fig. 3. Boxplot comparing DNA concentrations obtained by swabbing versus scraping for both bacterial and fungal samples.

Bacterial Beta Diversity Analysis PCoA based on the Jensen–Shannon distance matrix revealed substantial inter-individual differences in bacterial community compsosition. No distinct clustering was observed, indicating high beta diversity and personalized microbial signatures among patients, despite uniform anatomical sampling (Fig. 6).

Fungal sequencing and community structure
All 10 scraped samples yielded adequate fungal DNA for ITS sequencing. Shannon diversity ranged from 0.106 to 2.849, with a median of 0.9035 and an interquartile range (IQR) of 0.422 to 1.353, indicating moderate to high variation in fungal alpha diversity across individuals. N-M10 and N-M13 showed the highest diversity, while N-M3 had the lowest. Genus classification rates exceeded 97% in most samples (Table 2).

Malassezia dominated most samples, especially *M. restricta* and *M. globosa*. Other genera included *Candida*, *Rhodotorula*, and *Aureobasidium*, reflecting diverse fungal compositions across patients (Fig. 7).

Fungal Beta Diversity Analysis Distinct fungal compositions were observed between individuals, such as *Candida* predominance in N-M16 and diverse profiles in N-M10 and N-M13. These findings support the personalized nature of the skin mycobiome and suggest the utility of the scraping technique (Fig. 8).

To further evaluate the utility of the scraping method, we examined the relationship between DNA concentration and taxonomic diversity for both bacterial and fungal samples. Exploratory correlation analysis
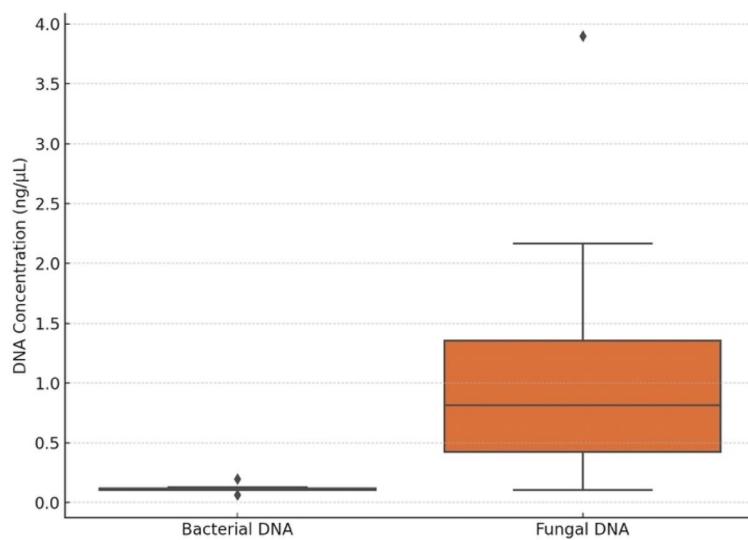
Fig. 4. Boxplot comparing bacterial and fungal DNA concentrations obtained by scraping. Fungal DNA yield was significantly higher than bacterial DNA yield across all samples.

Sample	OTUs	Shannon index	Genus classification (%)
VK-M1	18	0.35	99.98
VK-M2	208	1.30	99.73
VK-M3	8	0.20	99.99
VK-M8	6	0.25	99.95
VK-M9	11	0.03	99.92
VK-M10	13	0.10	99.99
VK-M11	30	0.03	99.97
VK-M13	325	2.85	99.95
VK-M16	6	0.70	99.98
VK-M17	45	0.50	99.94

Table 1. Bacterial DNA yield and diversity metrics in scraped samples.
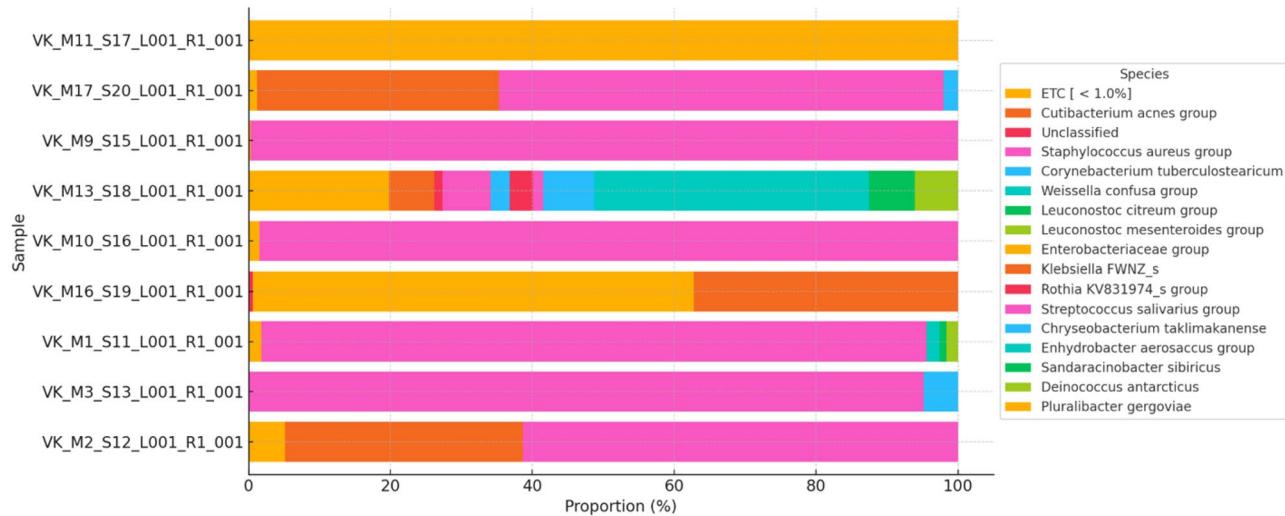
Fig. 5. Relative abundance of dominant bacterial species across 10 samples. Notably enriched taxa included *Staphylococcus aureus* group and *Cutibacterium acnes* group.
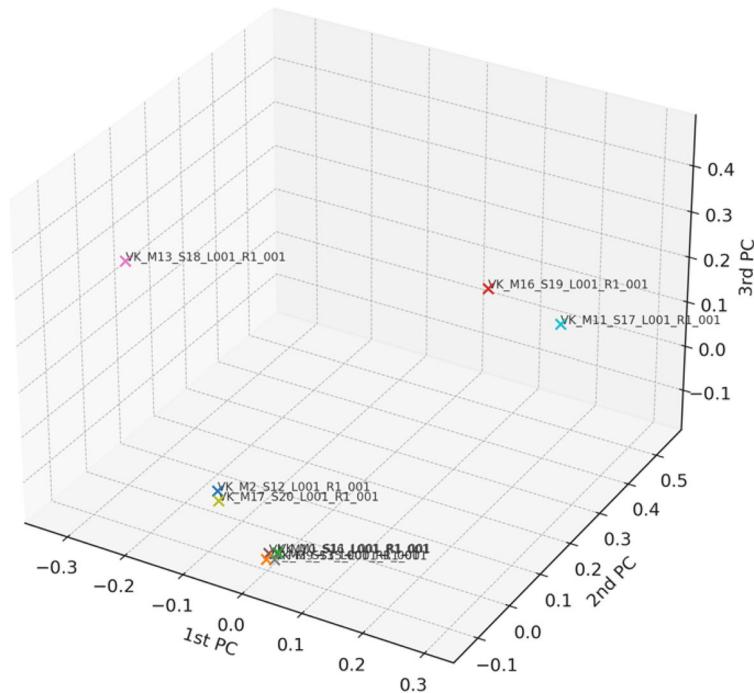
Fig. 6. PCoA plot using Jensen–Shannon distance matrix at the species level, including unclassified OTUs. Each dot represents an individual skin sample, demonstrating inter-individual beta diversity.

Sample	OTUs	Shannon index	Genus classification (%)
N-M1	85	1.132	97.89
N-M2	75	0.329	98.99
N-M3	54	0.106	99.34
N-M8	13	0.362	97.70
N-M9	67	1.410	98.98
N-M10	268	2.849	97.86
N-M11	50	0.675	99.47
N-M13	143	2.162	98.29
N-M16	40	1.182	37.51
N-M17	37	0.602	98.58

Table 2. Fungal DNA diversity metrics in scraped samples.

using Spearman's rank test revealed no significant association between bacterial DNA concentration and Shannon diversity index ($\rho = 0.21$, $p = 0.52$), suggesting that bacterial biomass alone does not predict community diversity. This may be attributable to dominant taxa such as *Cutibacterium acnes* or *Staphylococcus aureus* monopolizing the bacterial niche in certain individuals.

In contrast, fungal DNA concentration showed a moderate positive correlation with Shannon index ($\rho = 0.62$, $p = 0.047$), indicating that increased fungal biomass may support higher community richness and evenness (Fig. 9). Notably, samples N-M10 and N-M13 with the highest fungal DNA yields also exhibited the most diverse mycobiomes.

Furthermore, no correlation was found between bacterial and fungal Shannon indices across individuals ($\rho = -0.073$, $p = 0.841$), suggesting that bacterial and fungal communities are structured independently within the same host (Fig. 10).

Discussion

Sampling methodology plays a central role in the integrity of skin microbiome research, particularly in low-biomass or physiologically reactive environments such as sensitive facial skin. Previous studies have largely relied on swabbing small areas of the cheek (typically 2 cm × 2 cm), assuming this approach is both minimally invasive and sufficiently representative[1,5,10]. While these spot-based approaches are commonly used, our results indicate that even whole-face swabbing failed to yield measurable DNA, further emphasizing the limitations of swabbing in low-biomass, reactive skin conditions. Ogai et al.[12] compared swabbing, tape-stripping, and
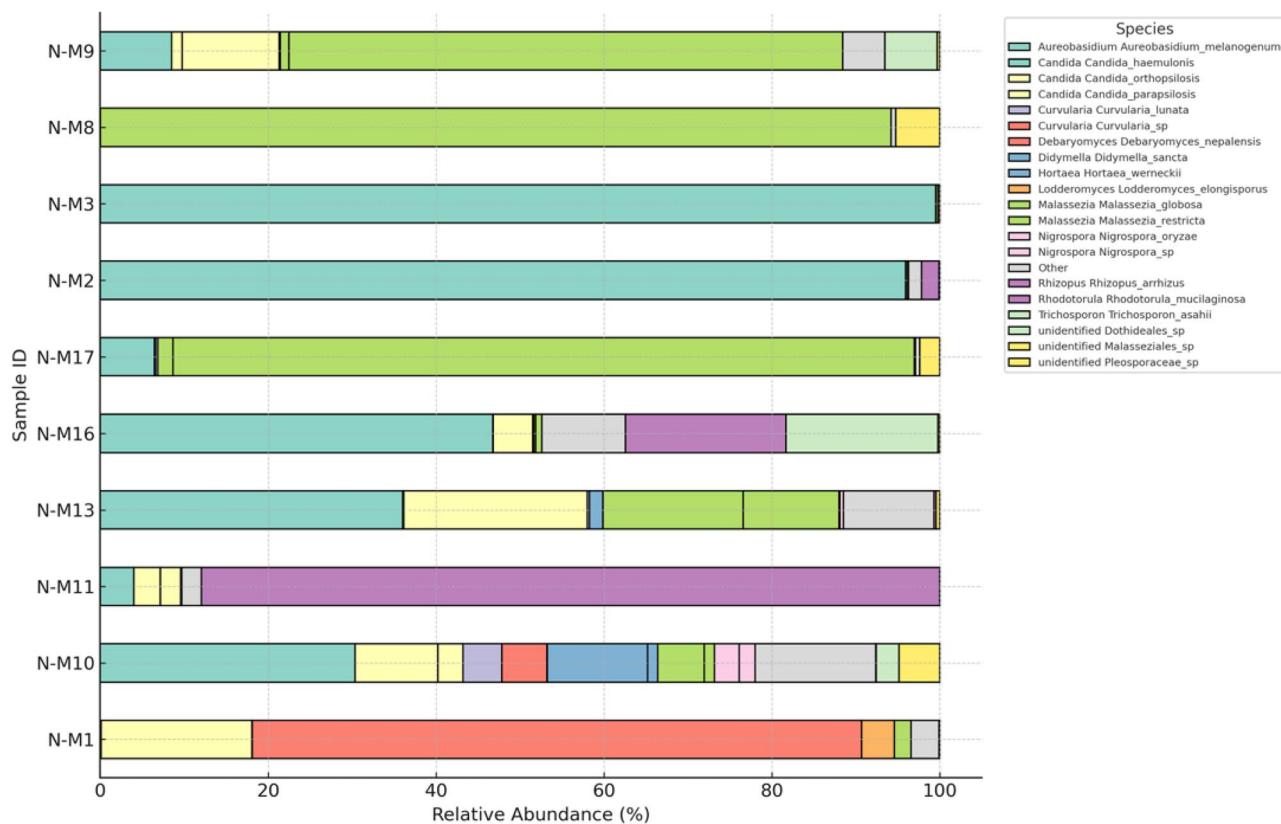
Fig. 7. Relative abundance of fungal species across individual patients, based on ITS sequencing data. The 20 most abundant taxa are shown, and all remaining species are grouped under "Other."

scraping, reporting that scraping yielded up to ten times more DNA and greater microbial diversity. Bouslimani et al.[10] further emphasized that limited-area swabbing may miss localized taxa due to the skin's topographical complexity, while Byrd et al.[5] highlighted how anatomical site, swab type, and pressure all influence microbial recovery and reproducibility.

In our study, even whole-face swabbing failed to yield detectable DNA, highlighting the method's limitation in real world sensitive skin contexts. This necessitated a second sampling session using an alternative approach. Scraping with a No. 10 surgical blade enabled reliable collection of stratum corneum material across the facial surface. The procedure was painless, non-invasive, and well-tolerated by all participants, with no visible skin damage noted in post-procedural photographs. All scraping sessions were completed within 20 - 30 minutes, without the need for repeated intervention, and reliably produced sufficient microbial DNA for sequencing and analysis in this pilot study.

These findings are consistent with prior studies reporting comparatively higher efficacy of scraping. Liu et al.[16] found higher DNA recovery from scraping compared to cotton swabs in seborrheic dermatitis patients, and Ogai et al.[12] reported similar results in healthy volunteers. Importantly, our study extends these conclusions to the context of sensitive skin, a population often excluded from microbiome sampling due to concerns over irritation or discomfort. Our data support that gentle scraping is safe, well-tolerated, and can yield sufficient material for bacterial and fungal profiling.

Beyond yield, the diversity and structure of the recovered microbial communities further validate the utility of scraping. Fungal DNA concentration correlated positively with Shannon diversity, whereas no such relationship was observed in bacterial samples. This aligns with findings by Findley et al.[20], who reported more heterogeneous and less predictable fungal communities compared to bacterial ones. The lack of correlation between bacterial and fungal alpha diversity also supports the view that these two microbial kingdoms are governed by distinct ecological mechanisms, an observation consistent with Xu et al.[8] and Kim et al.[6], who reported minimal concordance between bacterial and fungal signatures on the same skin site.

Beta diversity analyses further demonstrated the heterogeneity of microbial communities across individuals. Principal coordinates analysis (PCoA) based on Jensen-Shannon distances revealed substantial inter-individual variation in both bacterial and fungal composition, without clear clustering by any shared feature. This supports the personalized nature of the facial microbiome, even among individuals with the same clinical skin phenotype. These findings are consistent with Dreno et al.[7] and Kim et al.[6], who also reported high interpersonal variability in facial skin microbiota, particularly in sensitive skin populations.

All scraped samples yielded quantifiable bacterial and fungal DNA. Mean fungal DNA concentration exceeded bacterial DNA, possibly due to the structural resilience and biofilm-forming capacity of fungi such as
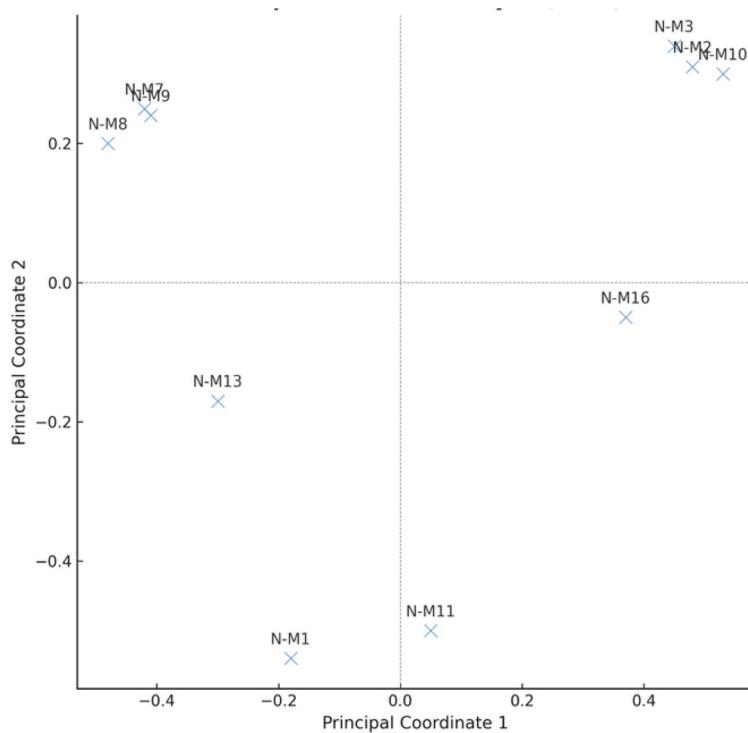
Fig. 8. PCoA of fungal beta diversity. Principal coordinate analysis (PCoA) based on Jensen–Shannon distances at the species level. Each dot represents a patient sample, revealing high inter-individual variability in the skin mycobiome.
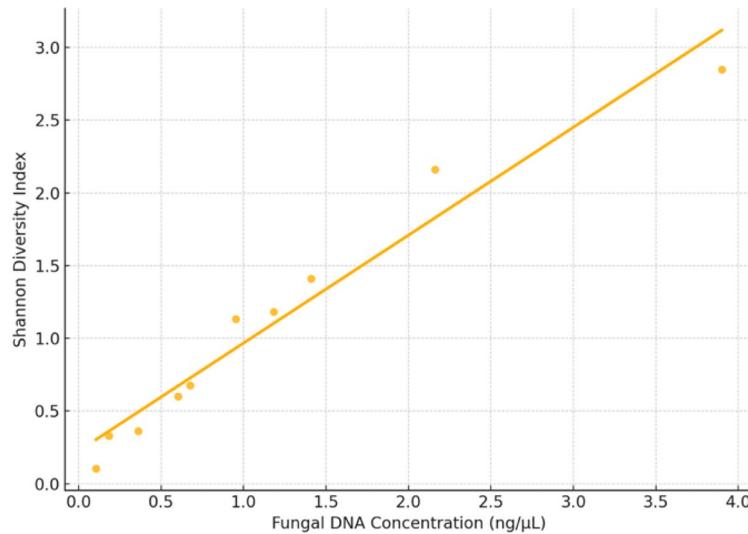

Fig. 9. Correlation between fungal DNA yield and fungal alpha diversity. Scatter plot showing a moderate positive correlation between fungal DNA concentration and Shannon index.

Malassezia[5,8]. Our findings are consistent with those of Findley et al.[20], who reported *Malassezia restricta* and *M. globosa* as the predominant fungi on adult facial skin, particularly in sebaceous areas.

Sequencing results reached high taxonomic resolution, with genus-level classification rates exceeding 99.7% for bacteria and 97% for fungi in 9 out of 10 samples. These values align with large-scale studies in both healthy and immunocompromised individuals[5,9,19]. For example, Jo et al.[9] demonstrated that scraping improved fungal recovery compared to swabbing in pediatric cohorts, while Oh et al.[19] emphasized that sequencing reliability was heavily dependent on DNA input quantity, reinforcing the strength of our approach.
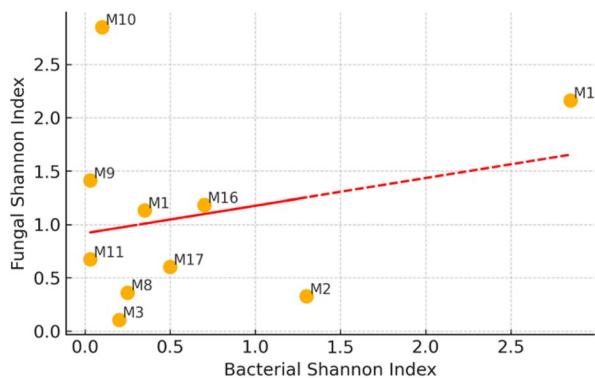
Fig. 10. Bacterial vs. fungal alpha diversity per patient. Shannon diversity indices of bacterial and fungal communities showed no significant correlation ($p = 0.841$), indicating independent community structures.

Shannon indices ranged from 0.03 to 2.85 for bacteria and 0.106 to 2.849 for fungi, highlighting broad inter-individual variability. VK-M13 exhibited the highest bacterial diversity (Shannon = 2.85; OTUs = 325), while N-M10 had the richest fungal community (Shannon = 2.849; OTUs = 268). In contrast, samples such as VK-M8 (bacteria) and N-M16 (fungi) displayed high DNA concentrations but low Shannon indices, suggesting microbial dominance by a few taxa, an observation that would likely be missed using conventional swabbing methods. These patterns mirror findings by Oh et al.[15], who reported temporal and individual variability in skin microbiomes, and Jo et al.[9], who noted age-related increases in fungal diversity.

In terms of bacterial composition, *Firmicutes* and *Proteobacteria* were dominant phyla, aligning with studies by Byrd et al.[5] and Dreno et al.[7]. At the species level, *Staphylococcus aureus group* and *Cutibacterium acnes group* were most prevalent. Interestingly, while Hillion et al.[1] observed *S. epidermidis* as dominant in both healthy and sensitive skin, our data suggested a relative shift toward *S. aureus* group, which may reflect subtle variations in barrier function or sampling depth. The predominance of *C. acnes group*, especially in sebaceous regions (e.g., VK-M8, VK-M9), supports its known role in maintaining skin homeostasis[7].

Fungal profiling revealed similar consistency with prior literature. *Malassezia* spp., especially *M. restricta* and *M. globosa*, were dominant in most individuals, as previously shown by Xu et al.[8] and Findley et al.[20]. However, increased abundance of *Candida parapsilosis* (N-M16) and *Rhodotorula mucilaginosa* (N-M11) highlights patient-specific mycobiome patterns. Such variation may reflect skin barrier dysfunction, topical product use, or environmental exposures, a concept supported by Kim et al.[3], who linked *Candida* expansion with immune alteration in sensitive skin.

Together, these findings suggest that sensitive skin does not harbor a universal dysbiotic signature but is instead shaped by individualized microbial profiles. This aligns with emerging perspectives in the skin microbiome field, which increasingly emphasize host-specific, environmental, and topographical factors over generalized disease-associated patterns[6,15]. It also underscores the value of methods like scraping, which can recover enough material to detect such personalized variation.

This pilot study suggests that gentle surgical scraping may represent a feasible and potentially more reliable alternative to swabbing for microbial DNA collection in sensitive skin. While swabbing failed even when applied across the full face, scraping enabled sequencing of both bacterial and fungal communities in this study. These findings reinforce the conclusions of Ogai et al.[12] and Byrd et al.[5] regarding the critical impact of sampling methodology on microbiome data quality. Moreover, our results extend these insights to the underrepresented population of sensitive skin individuals, suggesting that scraping is feasible, safe, and scientifically valuable for microbiome research.

Despite the promising findings, this study has several limitations. The sample size was small, and no healthy control group was included for comparison. However, as a proof-of-concept focused on methodological validation, the internal consistency across patients strengthens the evidence. Future research should explore longitudinal changes, correlate microbiome profiles with clinical features, and incorporate physiological parameters such as sebum content and skin barrier function to better understand host-microbe interactions in sensitive skin. Moreover, because this pilot study was specifically designed to compare sampling methods for subsequent sequencing, microbial culture and morphological colony data were not obtained. We acknowledge this as a limitation of the present work.

Conclusion

This pilot study suggests that gentle surgical scraping may provide a safe and potentially more reliable method than conventional swabbing for recovering microbial DNA from sensitive facial skin. The approach enabled sequencing of both bacterial and fungal communities and indicated the presence of notable inter-individual variability and individualized microbial patterns. These preliminary findings underscore the importance of sampling strategy in low-biomass skin studies, but larger and more comprehensive studies are needed to validate these observations and to further assess the role of scraping in dermatologic microbiome research, particularly in sensitive or clinically reactive skin types.

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Code availability

All custom scripts used for statistical analysis are available upon request.

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Author contributions

Thai Van Thanh Le: Supervision, dermatological expertise, clinical oversight, critical revision of the manuscript. The Bich Thanh Vuong: Conceptualization, methodology, patient recruitment and sampling, laboratory coordination, data analysis, manuscript drafting. Gia Hoang Linh Le: DNA extraction and optimization of sequencing workflow, technical troubleshooting, contribution to metagenomic data interpretation. Duc Minh Do : Bioinformatics analysis, statistical visualization, taxonomic profiling, manuscript editing. The Bich Thanh Vuong and Thai Van Thanh Le contributed equally to this work and share first authorship. All participants consented to the anonymous publication of their data and associated images.

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Competing interests

The authors declare no competing interests.

Ethics approval

The study protocol was reviewed and approved by the Institutional Review Board of the University of Medicine and Pharmacy at Ho Chi Minh City (Approval No. 382/HĐĐĐ-ĐHYD, dated 22 March 2023).

Informed consent

Written informed consent was obtained from all participants prior to enrollment.

Additional information

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