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Full-length 16S rRNA metabarcoding characterization of facial skin microbiota in acne patients: a case study in the Mekong Delta of Viet Nam

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Contributions: Xuong Mau Tu: conceptualization, software, data curation, formal analysis, interpretation of data, visualization, writing – original draft preparation; Pha Thi Nguyen: visualization, interpretation of results; Thanh Ngoc Nguyen: data curation, investigation, assistance in data interpretation; Lieu Thuy Thi Nguyen: formal analysis, validation, visualization, interpretation of data; Dung Ngoc Tran: supervision, data curation, visualization, critical revision of the manuscript; Phong Xuan Huynh: supervision, conceptualization, methodology, validation, writing – reviewing and editing. All authors contributed to the study conception and design, participated in data analysis or interpretation, and reviewed and approved the final version of the manuscript. All authors agree to be accountable for all aspects of the work.

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Abstract

Acne vulgaris is a chronic inflammatory disorder of the pilosebaceous unit in which skin microbiome dysbiosis plays a key pathogenic role. This study, based on full-length 16S rRNA gene amplicon sequencing (V1-V9), characterized facial microbial diversity in 45 participants classified as healthy (n=15), mild acne (n=15), and moderate-severe acne (n=15), using pooled samples for downstream microbiome analyses. Samples from the skin surface and sebaceous follicles were analyzed by 16S rRNA (V1-V9) sequencing using Illumina MiniSeq and processed *via* QIIME2. Alpha diversity (observed taxa, Shannon index), beta diversity (Bray-Curtis dissimilarity, permutational multivariate analysis of variance [PERMANOVA]), and biomarker taxa (linear discriminant analysis effect size [LEfSe]) were assessed. Bacillota, mainly *Staphylococcus* spp., predominated on the skin surface, with relative abundance increasing with acne severity, whereas follicles were dominated by *Cutibacterium acnes* (Actinomycetota). Follicular samples showed lower richness and Shannon diversity than surface samples, though intergroup differences were not significant. Principal coordinates analysis (PCoA) explained >65% of variation, revealing greater dispersion among surface communities but no clear clustering by severity (PERMANOVA $p>0.3$). LEfSe identified distinct bacterial biomarkers across clinical groups. Overall, site-specific microbial shifts – particularly *C. acnes* and *Staphylococcus* dysbiosis – appear central to acne development, suggesting microbiome-targeted interventions as potential therapeutic strategies.

Introduction

Acne vulgaris is a chronic inflammatory disease of the pilosebaceous unit, affecting approximately 80-85% of adolescents and young adults worldwide. Although not life-threatening, acne can lead to permanent scarring and may also negatively impact psychosocial well-being.[1] The pathogenesis of acne is complex, involving increased sebum production, follicular hyperkeratinization, inflammatory responses, and, importantly, the skin microbiota.[2]

Among common skin microorganisms, *Cutibacterium acnes* (formerly known as *Propionibacterium acnes*) is considered the most significant in acne. This bacterium contributes to lipid degradation, creates an anaerobic environment, and activates innate immune responses, resulting in localized inflammation.[3,4] However, the presence of *C. acnes* does not necessarily indicate disease, as it also colonizes healthy skin and even plays a protective role, such as producing fatty acids that maintain the skin's acidic pH.[5] Recent studies suggest that differences in *C. acnes* genotypes and strains (phylotypes) are decisive in pathogenesis: certain types are strongly associated with inflammatory acne, while others are prevalent

on healthy skin.[6,7] A recent genomic study on *C. acnes* emphasized the importance of distinguishing skin-colonizing *vs.* invasive lineages of *C. acnes* in acne pathophysiology, highlighting how strain-level differences reported in previous genomic studies may guide future therapeutic strategies.[8]

In addition to *C. acnes*, other microorganisms, such as *Staphylococcus epidermidis* and *Malassezia* spp., also play essential roles in skin homeostasis. Interactions between these species may help maintain a normal physiological state, and disruption of this balance may contribute to inflammation.[9,10] Accumulating evidence suggests dysbiosis of the facial skin microbiota as a hallmark of acne patients, characterized by altered microbial diversity and the predominance of bacterial strains harboring virulence-associated traits.[11-13] A systematic review confirmed that microbial shifts and reduced diversity are shared features of several dermatological disorders, including acne.[14]

Advances in 16S rRNA gene sequencing, particularly full-length 16S rRNA metabarcoding, have opened new avenues for taxonomic investigation of the skin microbiome. Compared with traditional culture-based methods, full-length 16S rRNA metabarcoding enables high-resolution taxonomic profiling of microbial communities, including uncultivable microorganisms, without providing direct functional or whole-genome information.[15,16] Applications of 16S rRNA gene-based microbiome analyses in acne research have produced novel findings, such as distinct microbial community structures between healthy and acne-affected skin, and the potential role of bacteriophages in modulating the skin microbiota.[7,11] For instance, previous microbiome studies, including shotgun metagenomic analyses, have demonstrated site-specific microbial signatures across different facial regions, providing additional insights into spatial variation in acne pathogenesis.[17] Moreover, recent work suggested that skincare products themselves may modulate the cutaneous microbiome, potentially influencing acne outcomes.[18]

Nevertheless, most of these studies have been conducted in Western populations, and data on the skin microbiome of acne patients in Viet Nam remain scarce. Therefore, the present study was undertaken to assess microbial diversity of facial skin in Vietnamese acne patients using full-length 16S rRNA metabarcoding to elucidate pathogenic mechanisms and provide a scientific foundation for novel microbiome-based therapeutic strategies.

Materials and Methods

Study site and period

The study was conducted at the Microbiology Laboratory, Institute of Biotechnology and Food Technology, Can Tho University, Viet Nam, between January 2025 and July 2025.

Subjects and sample size

A total of 45 participants were recruited, including 15 healthy individuals, 15 patients with mild acne, and 15 patients with moderate-severe acne. Diagnosis and severity grading followed the American Family Physician and DermNet New Zealand guidelines.[19] Participants receiving oral or topical antibiotics or probiotics at the time of sampling were excluded.[20] Additional exclusion criteria included immunosuppressive therapy, systemic metabolic disorders, or unwillingness to participate.

Sample collection

From each participant, two sample types were obtained: i) skin surface swabs, obtained by rubbing a moistened sterile swab across the cheek for 30 seconds; and ii) follicular material (comedonal content or sebum), collected with a sterile swab after brief local disinfection with 10% iodine alcohol (approximately 5 seconds), followed by gentle wiping with sterile physiological saline to remove residual disinfectant prior to sampling. Skin surface samples were always collected first, after participants refrained from facial washing for at least 5 hours, and disinfection was applied only after surface sampling was completed. Samples were preserved in 1.5 mL Eppendorf tubes containing DNA/RNA Shield (Zymo Research, USA) and stored at a low temperature until analysis. To minimize inter-individual variability and enhance group-level representativeness, samples were processed as pooled samples. Specifically, five individual samples of equal volume, from the same clinical group and sampling site (surface or follicle), were combined prior to DNA extraction. Accordingly, each clinical group (n=15 individuals) was represented by three pooled replicates per sampling site (n=3 pools/group/site), which constituted the statistical unit for all downstream analyses. This pooled-sample design was adopted to reduce inter-individual variability and to capture representative group-level microbial profiles. This approach maintains the average microbial composition of each group while reducing individual variation effects.

DNA extraction and sequencing

Genomic DNA was extracted using the DNeasy PowerSoil Pro Kit (Qiagen, USA). DNA quality and concentration were assessed by Qubit fluorometer and spectrophotometry. 16S rRNA amplicon libraries were prepared with the xGen 16S Amplicon Panel v2 Kit (IDT, USA) and quantified using the KAPA Library Quantification Kit (Illumina). Sequencing of the V1-V9 regions was performed on the Illumina MiniSeq platform.

Bioinformatics analysis

Sequencing data quality optimization: raw sequencing data (FAST5 files) generated by the Oxford Nanopore sequencing platform were basecalled using Dorado v7.6.7 (Oxford Nanopore Technologies [ONT]) to generate FASTQ files containing sequencing reads. The quality of sequencing reads was assessed using NanoQ v0.10.0. Adapter sequences and low-quality bases were filtered using Filtlong v0.2.1 (https://github.com/rrwick/Filtlong). All samples were processed using the same basecalling and quality-filtering pipeline to minimize potential batch effects. Raw reads were quality-filtered with Trimmomatic and Cutadapt to remove adapters, primers, and low-quality bases. Amplicon sequence variants were generated using the q2-DADA2 plugin in QIIME2.[21] Taxonomic assignment was performed against the SILVA v138 database with the q2-feature-classifier plugin.[22] Alpha diversity (observed taxa, Shannon index, rarefaction curves) and beta diversity (Bray-Curtis dissimilarity, principal coordinates analysis [PCoA]) were computed in QIIME2. Biomarker taxa were identified using the linear discriminant analysis effect size (LEfSe) algorithm.[23]

Statistical analysis

Data were analyzed using SPSS version 20 (analysis of variance [ANOVA], Kruskal-Wallis), R software (permutational multivariate analysis of variance [PERMANOVA]), and Microsoft Excel 2019 for visualization, with pooled samples treated as the unit of analysis. Results were expressed as mean ± standard deviation, and statistical significance was set at $p<0.05$.

Results and Discussion

Sequencing data quality optimization

Quality-filtered sequencing data showed consistent read length and quality across samples (Table 1), supporting the robustness of downstream diversity analyses.

Rarefaction curves

The rarefaction curve is a useful tool to determine whether sequencing depth is sufficient. This curve is generated by progressively subsampling sequencing data (through random removal) to lower depth levels, calculating alpha diversity at each step, and plotting diversity indices against the number of sequencing reads. Figure 1 presents the sequencing depth evaluation results, showing the increase in sequencing reads until reaching a saturation phase, where diversity indices no longer rise despite additional sequencing. The curves indicate that all samples have been sequenced at a sufficient depth.

The rarefaction curves of alpha diversity (observed features) indicate that most samples from both the skin surface and follicular regions reached a plateau, particularly at sequencing depths of approximately 30,000-45,000 reads, demonstrating that the sequencing depth was sufficient to adequately capture microbial diversity.[24] However, the degree of saturation was not completely uniform across clinical groups, with some samples from the healthy group tending to reach saturation earlier.

Comparison between sampling sites showed that the skin surface exhibited significantly higher observed features than the follicular region, reflecting greater microbial diversity on the skin surface. In contrast, the follicular microbiota displayed lower diversity, which may be associated with the distinct microenvironmental conditions of hair follicles, such as anaerobic conditions, lipid-rich substrates, and limited light exposure, favoring the growth of a restricted number of specialized bacterial taxa.[25]

In some samples, the rarefaction curves continued to increase slightly at higher read depths, suggesting the presence of rare taxa that may require deeper sequencing for comprehensive detection.[26] Overall, the similarity in rarefaction curve shapes across samples indicates consistent sample collection and processing procedures, supporting the comparability of microbial diversity analyses between groups.[27] The observed differences in richness are therefore likely to reflect ecological variation between skin niches, the presence of niche-specific taxa, and potential influences of sampling methodology.[28]

Microbial composition

Taxonomic analysis based on full-length 16S rRNA gene sequencing (V1-V9) revealed that the facial skin microbiome was primarily composed of two dominant phyla: Bacillota and Actinomycetota. Although other phyla, such as Pseudomonadota, Cyanobacteriota, and Deinococcota, were detected, their relative abundances were very low (<0.5%) and considered ecologically negligible. Importantly, there were striking differences in microbial community structure between the two sampling sites (Figure 2). On the skin surface, Bacillota predominated, and its proportion increased progressively with acne severity, from 39.41% in healthy controls to 65.37% in patients with mild acne, and 73.86% in those with moderate-severe acne. In contrast, Actinomycetota decreased from 60.35% in healthy subjects to 25.75% in those with severe acne. At the genus level, *Staphylococcus* spp. dominated Bacillota, while *C. acnes* was the most abundant member of Actinomycetota. Conversely, in sebaceous follicles, Actinomycetota consistently predominated (72.34% in healthy and 72.40% in severe acne samples), whereas Bacillota accounted for only 27.18-40.26%. This highlights a clear ecological partition, Bacillota/*Staphylococcus* are surface-associated, while Actinomycetota/*C. acnes* predominate in the anaerobic, lipid-rich follicular niche.

Our findings revealed a clear ecological divide between the skin surface and sebaceous follicles. The surface was dominated by Bacillota, especially *Staphylococcus* spp., whereas follicles were enriched with Actinomycetota, particularly *C. acnes*. This distribution reflects physiological differences: the oxygen-exposed, variable surface favors aerobic bacteria, while follicles are anaerobic and lipid-rich, providing an ideal niche for *C. acnes*.[3,29] Recent studies have reported that certain *C. acnes* lineages are associated with virulence-related traits, potentially contributing to a shift from a commensal to a pathogenic role under dysbiotic conditions.[30] Because samples were pooled prior to sequencing, the findings reflect group-level microbial patterns and do not allow inference on inter-individual variability. Moreover, brief disinfection of the skin prior to follicular sampling may have reduced certain surface-associated microorganisms, potentially influencing the detected follicular microbial profiles.

Alpha diversity

Alpha diversity analyses based on pooled samples indicated differences in richness and evenness at the group level across sampling sites and clinical categories (Figure 3). On the surface, richness (observed taxa) was higher in pooled samples from mild (39.67) and moderate-severe (36.67) acne compared with healthy controls (25.00), although the differences were not statistically significant (Kruskal-Wallis, $p=0.337$). No statistically significant differences were detected among pooled samples across clinical groups (Kruskal-Wallis test, $p>0.05$). In follicles, pooled samples from healthy controls showed the highest richness (17.33), whereas mild and moderate-severe acne samples exhibited lower values (9.00 and 10.00, respectively), although the differences were not significant (ANOVA, $p=0.71$). Between-site comparisons showed significantly higher richness on the surface than in follicles for both mild ($p=0.046$) and moderate-severe acne ($p=0.021$) (Figure 3A). Shannon diversity indices calculated from pooled samples supported this pattern; in healthy controls, Shannon values were comparable between surface and follicular sites, whereas in acne groups, Shannon indices were consistently higher in surface samples, consistent with follicular dysbiosis characterized by increased dominance of *C. acnes* (Figure 3B). Overall, alpha diversity patterns derived from pooled samples suggest site-specific rather than severity-driven differences, with reduced richness and evenness observed in follicular communities compared with surface communities at the group level.

Richness and Shannon diversity indices indicated reduced diversity in follicles of acne patients, although intergroup differences were not statistically significant. This suggests that dysbiosis is more site-specific than global. Similar conclusions were reported by Fitz-Gibbon *et al.* (2013) and Barnard *et al.* (2016), emphasizing the importance of strain-level variation of *C. acnes*.[6,11] Recent studies have also

reported that acne duration shapes microbiota assembly processes, with long-standing acne communities more influenced by stochastic drift, reducing stability.[31]

Beta diversity

Beta diversity, assessed by PCoA using Bray-Curtis dissimilarity, further illustrated these differences (Figure 4). On the surface, the first two axes explained 67.14% of the variation (axis 1: 39.29%; axis 2: 27.85%), with broader sample dispersion indicating greater variability among pooled samples (Figure 4A). In follicles, the first two axes explained 71.28% of variation (axis 1: 43.03%; axis 2: 28.25%), with tighter clustering of samples (Figure 4B). Despite these visual patterns, PERMANOVA performed on pooled samples indicated that differences among clinical groups were not statistically significant (surface: R^2=0.291, p=0.304; follicles: R^2=0.095, p=0.964). Dispersion tests were likewise non-significant (p=0.319 and 0.78, respectively). These findings suggest that acne severity is not strongly associated with global microbiome restructuring, but rather with site-specific alterations in community composition.

PCoA and PERMANOVA did not reveal significant global differences, suggesting that acne pathogenesis may be driven less by wholesale microbiome restructuring and more by the expansion of pathogenic *C. acnes* lineages. This is consistent with Barnard *et al.* (2016), who note that the overall skin microbiome structure remains relatively stable,[11] and the recent consensus that strain-level composition and virulence-associated traits change significantly.[32] Thus, acne exemplifies a quality-over-quantity mechanism, in which strain-level differences and previously reported virulence-associated traits may be more relevant than global diversity metrics.

Biomarker taxa (LEfSe)

LEfSe analysis identified group-specific microbial biomarkers (Figure 5). Given the pooled-sample design and the limited number of pooled replicates per group, LEfSe results should be interpreted as exploratory and reflective of group-level differences rather than individual-level biomarkers. In healthy controls, taxa such as *Cutibacterium modestum* and *S. epidermidis* were enriched, which have been previously reported to be associated with skin health and commensal protective roles. In mild acne samples, *C. acnes* was markedly increased along with certain *Staphylococcus* strains. In moderate-to-severe acne samples, anaerobic genera including *Anaerococcus*, *Peptoniphilus*, and *Finegoldia* were enriched. These taxa have been previously reported to be associated with inflammatory processes and tissue damage in acne and other skin infections. These results suggest that, at the group level, acne-

associated microbial patterns may involve not only increased abundance of *C. acnes* but also the co-occurrence of opportunistic anaerobes.

LEfSe analysis highlighted taxa enriched at the group level, including *S. epidermidis* and *C. modestum*, enriched in healthy skin, which are potentially protective, while *C. acnes* and opportunistic anaerobes (*Anaerococcus*, *Peptoniphilus*, and *Finegoldia*) were enriched in acne patients. These findings are consistent with the framework proposed by O'Neill and Gallo (2018), who suggested that acne pathogenesis involves complex microbial interactions between *C. acnes* and co-occurring anaerobes.[12] Recent clinical trials testing topical probiotics have demonstrated improvements in both microbiota balance and acne severity.[33] Moreover, reviews have emphasized the therapeutic potential of microbiome modulation, including probiotics, phage therapy, and photodynamic therapy, as promising alternatives to antibiotics.[34,35]

Conclusions

This study demonstrates a distinct ecological divide of the facial skin microbiome: Bacillota (*Staphylococcus* spp.) predominated on the skin surface, whereas Actinomycetota, mainly *C. acnes*, dominated sebaceous follicles. Alpha diversity was reduced in the follicles of acne patients, with lower Shannon indices compared to surface samples. Although beta diversity analysis did not reveal significant global differences across clinical groups, LEfSe identified enriched anaerobic taxa in moderate-severe acne. Collectively, these findings indicate that acne vulgaris is primarily associated with site-specific dysbiosis within sebaceous follicles, rather than broad alterations of the surface microbiota. Future studies should investigate strain-level variation of *C. acnes* to clarify pathogenic *vs.* commensal lineages. Incorporating functional metagenomics would help elucidate strain-level functional attributes, including virulence-associated gene repertoires and metabolic capabilities of skin microbes. Clinically, the results support the exploration of microbiome-targeted interventions, including topical probiotics, bacteriophage therapy, and ecological modulators to restore follicular microbial balance for acne management. This study is subject to certain limitations inherent to full-length 16S rRNA gene metabarcoding. While this approach enables robust, high-resolution taxonomic profiling of skin microbiota, it does not permit direct assessment of gene content, functional potential, or metabolic pathways. Consequently, the associations discussed here are based on taxonomic and ecological patterns rather than functional inference. Future investigations incorporating shotgun metagenomics and/or metatranscriptomics will be necessary to resolve strain-level diversity and clarify the functional mechanisms involved in acne pathogenesis.

References

1. Preneau S, Dreno B. Female acne - a different subtype of teenager acne? J Eur Acad Dermatol Venereol 2012;26:277-82.

2. Guo Z, Yang Y, Wu Q, et al. New insights into the characteristic skin microorganisms in different grades of acne and different acne sites. Front Microbiol 2023;14:1167923.

3. Dreno B, Pecastaings S, Corvec S, et al. *Cutibacterium acnes* (*Propionibacterium acnes*) and acne vulgaris: a brief look at the latest updates. J Eur Acad Dermatol Venereol 2018;32:5-14.

4. Spittaels KJ, Ongena R, Zouboulis CC, et al. *Cutibacterium acnes* phylotype I and II strains Interact differently with human skin cells. Front Cell Infect Microbiol 2020;10:575164.

5. Byrd AL, Belkaid Y, Segre JA. The human skin microbiome. Nat Rev Microbiol 2018;16:143-55.

6. Fitz-Gibbon S, Tomida S, Chiu BH, et al. *Propionibacterium acnes* strain populations in the human skin microbiome associated with acne. J Invest Dermatol 2013;133:2152-60.

7. McLaughlin J, Watterson S, Layton AM, et al. *Propionibacterium acnes* and acne vulgaris: New insights from the integration of population genetic, multi-omic, biochemical and host-microbe studies. Microorganisms 2019;7:128.

8. Brüggemann H, Salar-Vidal L, Gollnick HPM, Lood R. A janus-faced bacterium: Host-beneficial and - detrimental roles of *Cutibacterium acnes*. Front Microbiol 2021;12:673845.

9. Dagnelie MA, Corvec S, Saint-Jean M, et al. *Cutibacterium acnes* phylotypes diversity loss: A trigger for skin inflammatory process. J Eur Acad Dermatol Venereol 2019;33:2340-8.

10. Niedźwiedzka A, Micallef MP, Biazzo M, Podrini C. The role of the skin microbiome in acne: Challenges and Future therapeutic opportunities. Int J Mol Sci 2024;25:11422.

11. Barnard E, Shi B, Kang D, et al. The balance of metagenomic elements shapes the skin microbiome in acne and health. Sci Rep 2016;6:39491.

12. O'Neill AM, Gallo RL. Host-microbiome interactions and recent progress into understanding the biology of acne vulgaris. Microbiome 2018;6:177.

13. Kurokawa I, Layton AM, Ogawa R. Updated treatment for acne: Targeted therapy based on pathogenesis. Dermatol Ther 2021;11:1129-39.

14. Lee CH, Min M, Jin SS, Sivamani RK. Skin microbiome shifts in various dermatological conditions. J Clin Med 2025;14:6137.

15. Grice EA, Segre JA. The skin microbiome. Nat Rev Microbiol 2011;9:244-53.

16. Regueira-Iglesias A, Balsa-Castro C, Blanco-Pintos T, Tomás I. Critical review of 16S rRNA gene sequencing workflow in microbiome studies: From primer selection to advanced data analysis. Mol Oral Microbiol 2023;38:347-99.
17. Wei Q, Li Z, Gu Z, et al. Shotgun metagenomic sequencing reveals skin microbial variability from different facial sites. Front Microbiol 2022;13:933189.
18. Mim MF, Sikder MH, Chowdhury MZH, et al. The dynamic relationship between skin microbiomes and personal care products: A comprehensive review. Heliyon 2024;10:e34549.
19. Dermnet. Acne vulgaris. 2014. Available from: https://dermnetnz.org/topics/acne-vulgaris
20. Ogai K, Nagase S, Mukai K, et al. A Comparison of Techniques for Collecting Skin Microbiome Samples: Swabbing Versus Tape-Stripping. Front Microbiol 2018;9:2362.
21. Callahan BJ, McMurdie PJ, Rosen MJ, et al. DADA2: High-resolution sample inference from Illumina amplicon data. Nat Methods 2016;13:581-3.
22. Quast C, Pruesse E, Yilmaz P, et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res 2013;41:D590-6.
23. Segata N, Izard J, Waldron L, et al. Metagenomic biomarker discovery and explanation. Genome Biol 2011;12:R60.
24. Li M, Kopylova E, Mao J, et al. Microbiome and lipidomic analysis reveal the interplay between skin bacteria and lipids in a cohort study. Front Microbiol 2024;15:1383656.
25. Grice EA, Kong HH, Conlan S, et al. Topographical and temporal diversity of the human skin microbiome. Science 2009;324:1190-2.
26. MacGibeny MA, Adjei S, Pyle H, et al. The skin microbiome in dermatologic disease. J Am Acad Dermatol 2025;93:339-48.
27. Sun C, Hu G, Yi L, et al. Integrated analysis of facial microbiome and skin physio-optical properties unveils cutotype-dependent aging effects. Microbiome 2024;12:163.
28. Pérez-Losada M, Crandall KA. Spatial diversity of the skin bacteriome. Front Microbiol 2023;14:1257276.
29. Kurokawa I, Danby FW, Ju Q, et al. New developments in our understanding of acne pathogenesis and treatment. Exp Dermatol 2009;18:821-32.
30. Chen Q, Liu C, Tao J, et al. Insights into microbial dysbiosis and *Cutibacterium acnes* CAMP factor interactions in acne vulgaris. Microb Genom 2025;11:001449.
31. Sun L, Wang Q, Huang J, et al. Disrupting the balance: how acne duration impacts skin microbiota assembly processes. Microbiol Spectr 2025;13:e0260324.

32. Dessinioti C, Katsambas A. The microbiome and acne: perspectives for treatment. Dermatol Ther 2024;14:31-44.
33. Biazzo M, Pinzauti D, Podrini C. SkinDuo(TM) as a targeted probiotic therapy: Shifts in skin microbiota and clinical outcomes in acne patients. Int J Mol Sci 2025;26:5000.
34. Huang C, Zhuo F, Han B, et al. The updates and implications of cutaneous microbiota in acne. Cell Biosci 2023;13:113.
35. Raza ML, Ali SI, Bhojani A, et al. Microbiome modulation in dermatological disorders: Current insights and therapeutic prospects - A narrative review. Microb Pathog 2025;208:107940.

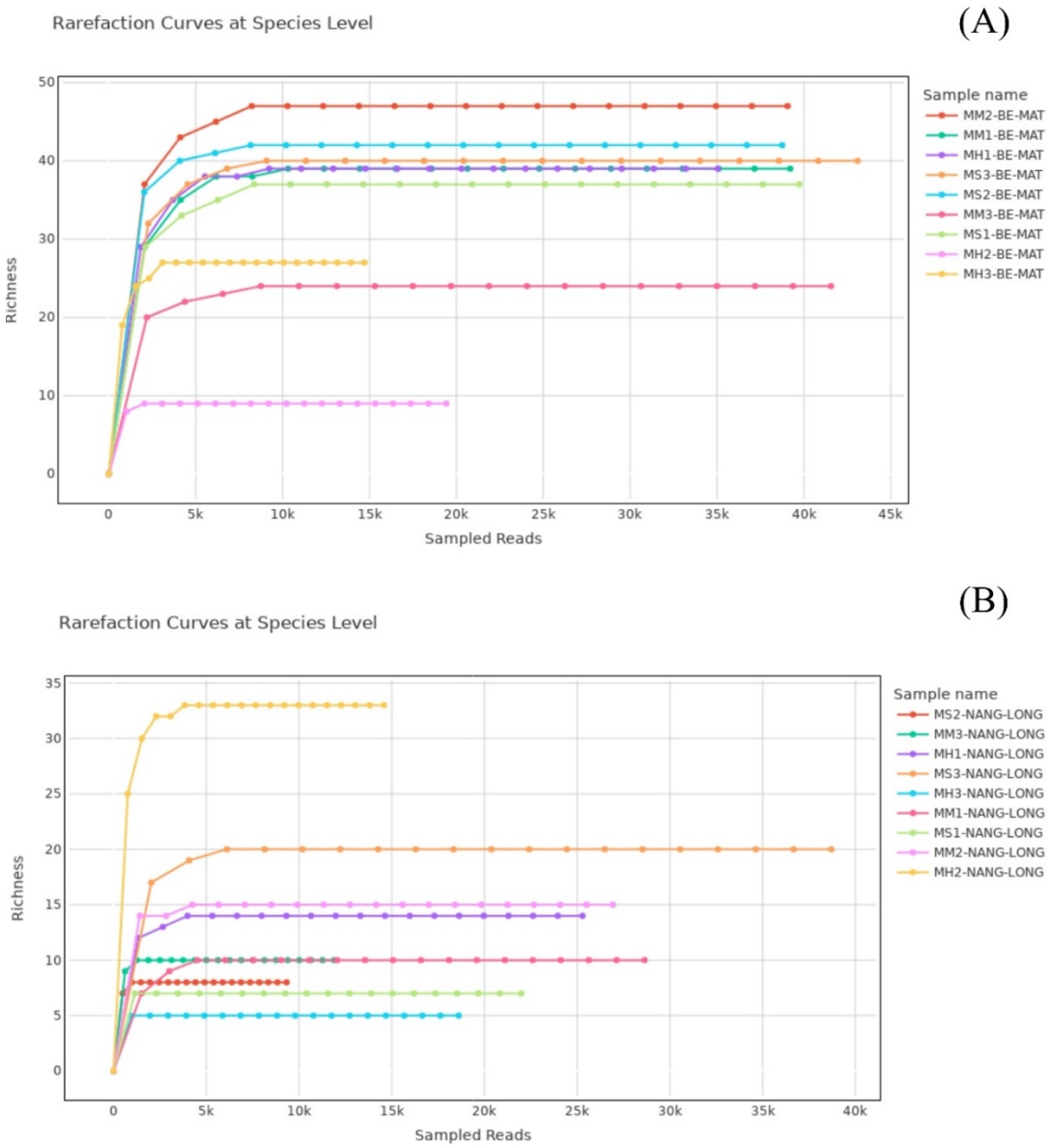
Table 1. Sequence quality metrics.

No.	FASTQ file	Total reads	Total bases	Average read length	Average quality score	%>Q10
1	MH3-BE-MAT	16,612	23,881,980	1,437.60	12.40	82.9
2	MH3-BE-MAT-passed	14,702	22,895,587	1,557.30	12.50	84.2
3	MM3-BE-MAT	47,051	71,023,817	1,509.50	12.70	86.3
4	MM3-BE-MAT-passed	43,974	67,954,079	1,545.30	12.80	87.1
5	MM2-BE-MAT	48,066	69,577,324	1,447.50	12.60	86.1
6	MM2-BE-MAT-passed	44,417	66,418,500	1,495.30	12.70	86.9
7	MH1-BE-MAT	43,948	63,449,758	1,443.70	12.60	85.8
8	MH1-BE-MAT- passed	40,002	59,962,776	1,499.00	12.70	86.6
9	MS2-BE-MAT	41,829	64,200,884	1,534.80	12.70	86.0
10	MS2-BE-MAT-passed	39,716	61,907,474	1,558.80	12.80	86.7
11	MS3-BE-MAT	48,001	72,443,635	1,509.20	12.70	86.7
12	MS3-BE-MAT- passed	44,665	69,660,599	1,559.60	12.80	87.5
13	MH2-BE-MAT	20,411	31,620,493	1,549.20	12.70	86.7
14	MH2-BE-MAT-passed	19,592	30,819,879	1,573.10	12.70	87.1
15	MS1-BE-MAT	45,164	67,308,731	1,490.30	12.70	85.1
16	MS1-BE-MAT- passed	42,032	64,202,892	1,527.50	12.80	86.1
17	MM1-BE-MAT	40,846	63,996,537	1,566.80	12.80	86.9
18	MM1-BE-MAT-passed	39,230	62,135,483	1,583.90	12.90	87.5
19	MM3-NANG-LONG	16,094	20,094,297	1,248.60	12.00	79.2
20	MM3-NANG-LONG-passed	12,400	18,511,343	1,492.90	12.10	80.5
21	MM1-NANG-LONG	31,047	46,070,709	1,483.90	12.10	84.4
22	MM1-NANG-LONG-passed	28,638	44,667,021	1,559.70	12.20	85.2
23	MM2-NANG-LONG	32,954	44,220,163	1,341.90	12.00	79.8
24	MM2-NANG-LONG-passed	27,232	41,562,484	1,526.20	12.20	81.2
25	MS2-NANG-LONG	17,196	20,494,298	1,191.80	12.10	79.8
26	MS2-NANG-LONG-passed	13,692	18,321,348	1,338.10	12.20	80.9
27	MH2-NANG-LONG	17,664	23,865,678	1,351.10	12.10	80.8
28	MH2-NANG-LONG-passed	14,587	22,430,968	1,537.70	12.20	82.3
29	MH1-NANG-LONG	29,109	40,866,615	1,403.90	12.00	80.3
30	MH1-NANG-LONG-passed	25,290	38,954,874	1,540.30	12.20	81.7
31	MS1-NANG-LONG	36,609	45,449,563	1,241.50	11.90	78.0

32	MS1-NANG-LONG-passed	30,247	41,622,107	1,376.10	12.00	79.4
33	MS3-NANG-LONG	40,587	62,223,270	1,533.10	12.10	84.8
34	MS3-NANG-LONG -passed	38,742	60,937,950	1,572.90	12.20	85.6
35	MH3-NANG-LONG	20,466	29,815,531	1,456.80	12.10	83.9
36	MH3-NANG-LONG-passed	18,611	28,891,984	1,552.40	12.10	84.8

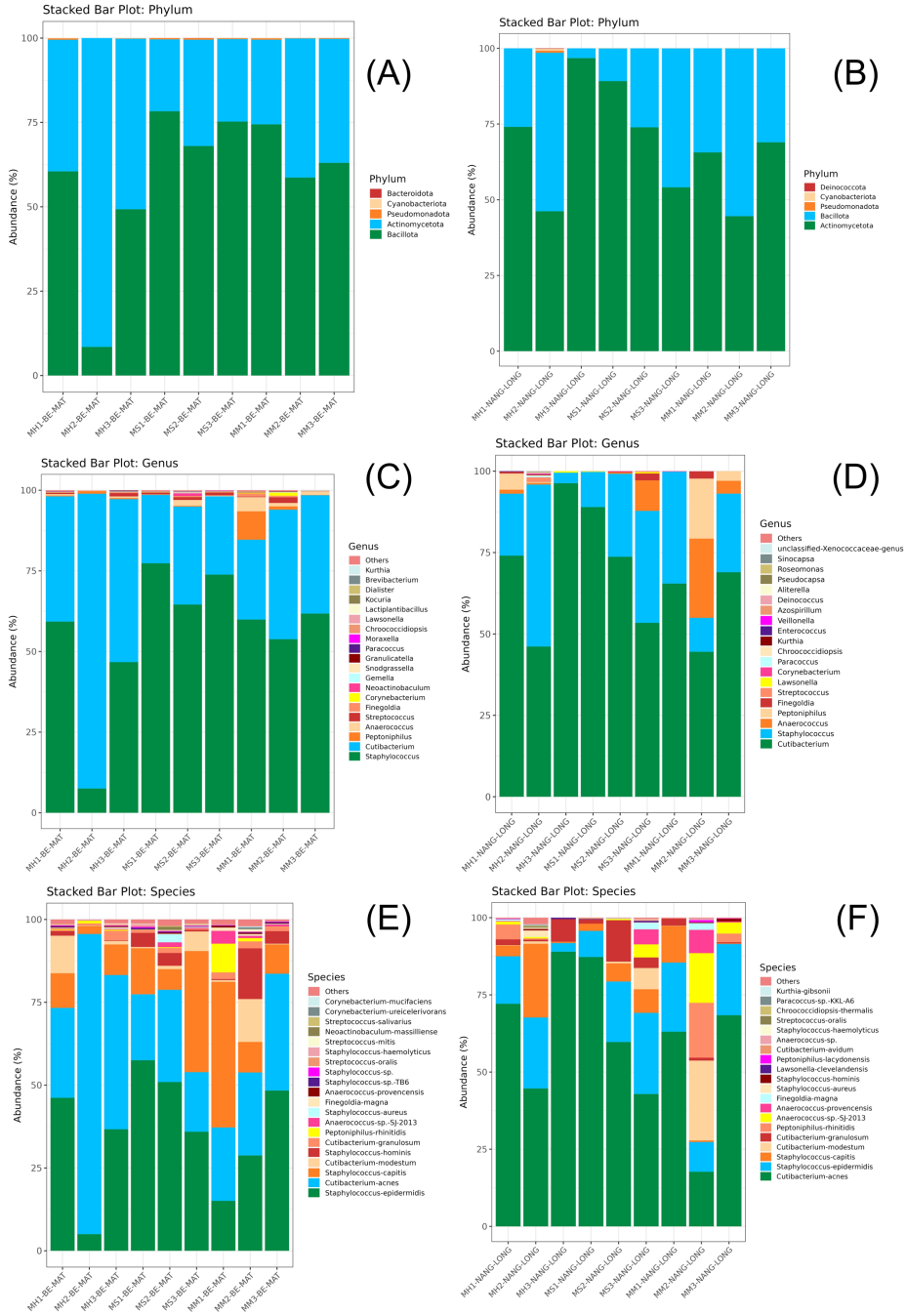
Samples are paired and arranged accordingly. For example, Sample A is the original sequenced sample, while Sample A-passed is Sample A after removing low-quality reads. Total bases: the total number of bases sequenced for each sample. %>Q10: the percentage of bases with a quality score (Qscore) greater than 10 out of the total sequenced bases. MH, healthy group; MM, mild acne group; MS, moderate-to-severe acne group; BE-MAT, surface region; NANG-LONG, follicular region.

Figure 1. Rarefaction curves at the species level in (**A**) surface and (**B**) follicular regions. Each curve represents a sample. The X-axis corresponds to the number of sequencing reads, while the Y-axis represents the value of the alpha diversity, measured as Observed Features.



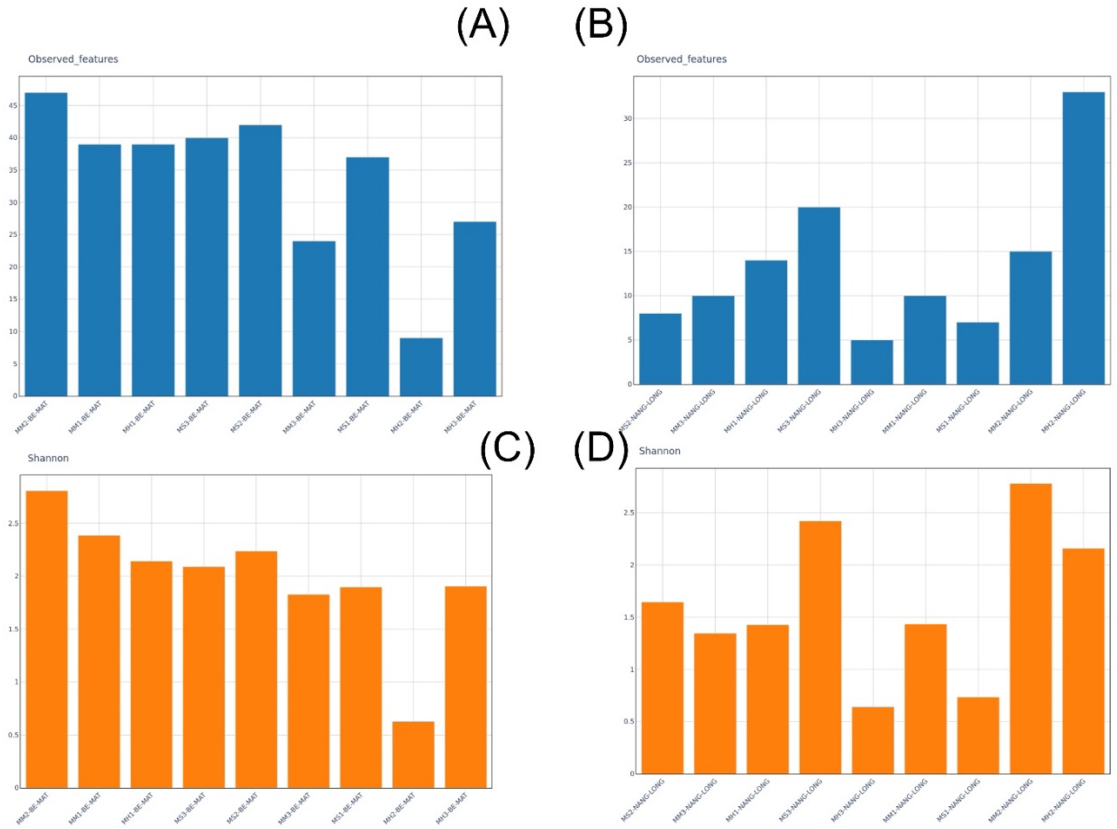
MH, healthy group; MM, mild acne group; MS, moderate-to-severe acne group; BE-MAT, surface region; NANG-LONG, follicular region.

Figure 2. Relative abundance of microbial phylum in (**A**) surface and (**B**) follicular regions, of microbial genus in (**C**) surface and (**D**) follicular regions, and of microbial genus in (**E**) surface and (**F**) follicular regions.



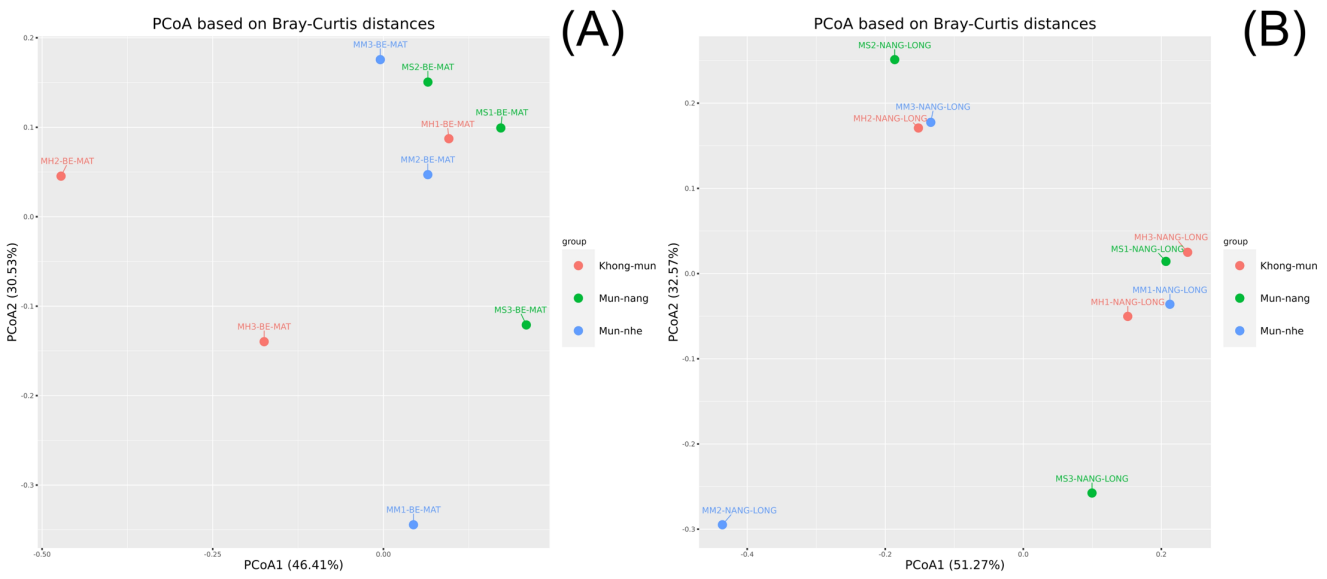
MH, healthy group; MM, mild acne group; MS, moderate-to-severe acne group; BE-MAT, surface region; NANG-LONG, follicular region.

Figure 3. Alpha diversity indices, including observed features and Shannon index in (**A**) surface and (**B**) follicular regions.



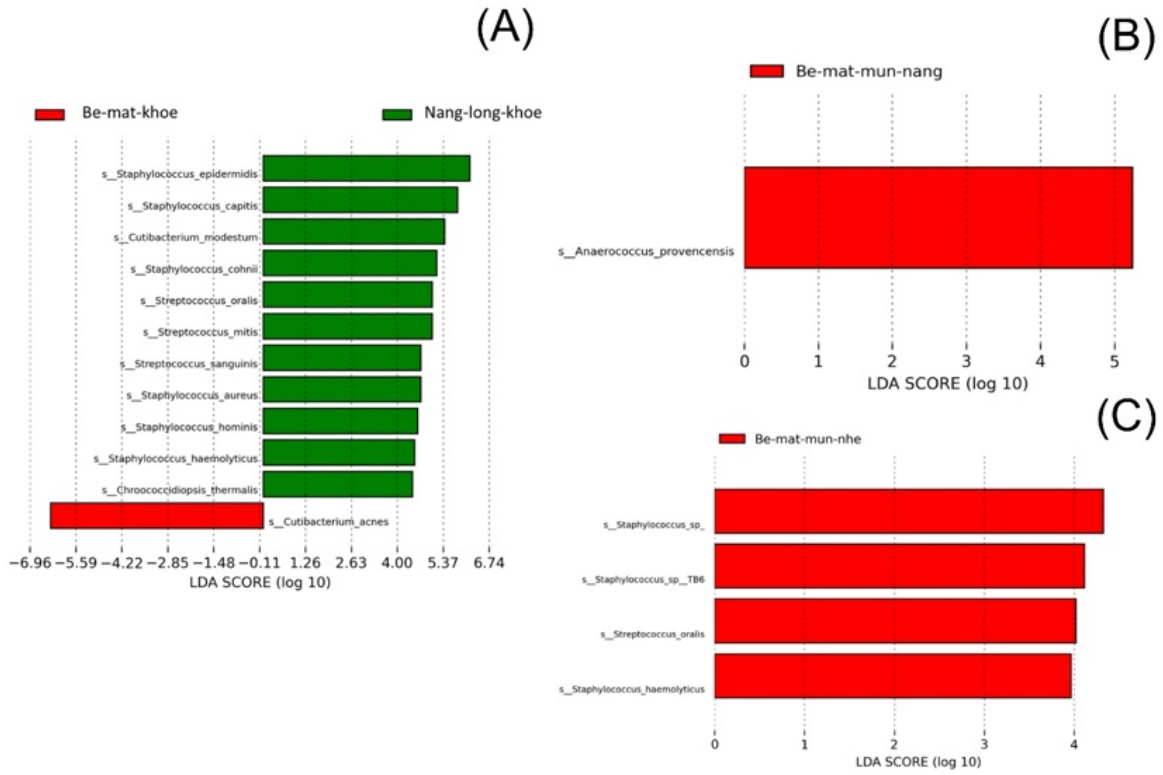
MH, healthy group; MM, mild acne group; MS, moderate-to-severe acne group; BE-MAT, surface region; NANG-LONG, follicular region.

Figure 4. PCoA based on the Bray-Curtis distance matrix among study groups in (**A**) surface and (**B**) follicular regions.



MH, healthy group; MM, mild acne group; MS, moderate-to-severe acne group; BE-MAT, surface region; NANG-LONG, follicular region.

Figure 5. Biomarker taxa identified at the species level among surface and follicular samples: (**A**) healthy group; (**B**) mild acne group; and (**C**) moderate-to-severe acne group.



LDA, linear discriminant analysis.