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Comparative Analysis of Skin Microbiome in Acne Lesions and Healthy Skin Using 16S rRNA Gene Sequencing

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Abstract

Acne vulgaris (AV) is a common dermatological disorder in adolescents, encompassing both non-inflammatory and inflammatory lesions, with growing evidence implicating the skin microbiome in its pathogenesis. This study analyzed skin lesion samples from 12 adolescents with AV using 16S rRNA high-throughput sequencing, with 12 healthy skin microbiome datasets as references. A total of 4.7 million high-quality reads were obtained, yielding 765,211 clean reads clustered into 1013 operational taxonomic units (OTUs). Microbial communities in lesions differed markedly from those in healthy skin. At the phylum level, lesions showed higher proportions of *Bacteroidota* and *Bacillota*, whereas healthy skin was dominated by *Actinobacteria*. At the genus level, lesions were modestly but significantly higher in *Staphylococcus*, *Corynebacterium*, and *Peptoniphilus*, while *Cutibacterium* was more abundant in healthy skin. Alpha diversity analysis revealed greater species richness and phylogenetic diversity in healthy skin, but higher evenness in lesions. Beta diversity confirmed significant differences in community structure. Functional prediction identified 391 metabolic pathways, 163 of which differed significantly; only three were enriched in lesions, while 160 were more abundant in healthy skin. Lipase activity was elevated in lesions, whereas hyaluronate lyase activity was higher in healthy skin. These findings indicate that healthy skin supports a richer and more functionally diverse microbial metabolism, whereas acne lesions are associated with reduced metabolic capabilities. Overall, the acne lesion microbiome exhibits reduced diversity, altered bacterial composition, and distinct functional traits compared to healthy skin, underscoring the role of microbial imbalance in acne and suggesting potential microbial targets for treatment.

Keywords: *Cutibacterium acnes*; follicular content; skin dysbiosis

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1. Introduction

Acne vulgaris is a common inflammatory disorder of the pilosebaceous unit that disproportionately affects adolescents and can significantly impair quality of life. Microbial dysbiosis is increasingly recognized as a key contributor to acne pathogenesis. Rather

than a uniform pathogen, *Cutibacterium acnes* comprises multiple phylotypes with distinct pathogenic potential—some enriched in healthy skin and others associated with inflamed lesions [1]. This shift in understanding has moved research beyond simple quantification of *C. acnes* toward examining strain-level diversity and ecological interactions within the skin microbial community.

The pilosebaceous unit hosts a complex network of microbes, including *Staphylococcus epidermidis*, *Corynebacterium species*, and *Malassezia fungi*, which can modulate inflammation, sebum metabolism, and host immune responses [2,3]. Environmental exposures—such as humidity, pollution, antibiotic use, and skincare products—further shape microbial composition and may exacerbate dysbiosis [4,5]. These factors are particularly relevant in tropical regions, where high humidity and increased sebum activity create a distinct cutaneous microenvironment that may influence microbial growth, competition, and virulence. Despite this, microbiome studies in tropical adolescent populations remain limited.

Metagenomics refers to whole-genome shotgun sequencing, which provides comprehensive insight into both taxonomic composition and functional potential. In contrast, 16S rRNA gene profiling is a targeted amplicon-based method that characterizes bacterial community structure using conserved ribosomal gene regions. The present study employs 16S rRNA gene sequencing—rather than whole-genome metagenomics—to examine microbial community differences between acne lesions and healthy skin. Although 16S-based approaches primarily provide taxonomic resolution, advances in bioinformatics now allow predictive functional profiling, offering insight into potential microbial activities that may contribute to acne pathophysiology [6,7].

Most existing acne microbiome studies have focused on Western populations, leaving important gaps in understanding microbial variation across climatic and ethnic backgrounds. Given that warmer climates, higher baseline sebum production, and unique cultural skincare practices can influence microbial ecology, targeted research in tropical settings is essential for identifying population-specific microbial signatures and functional pathways [8].

In this study, we used 16S rRNA gene sequencing to compare the skin microbiome of acne lesions and healthy controls in a tropical adolescent population. Predictive functional profiling was applied to explore potential microbial metabolic pathways associated with acne, providing both compositional and functional insights into acne-related dysbiosis.

2. Materials and Methods

2.1. Study Population and Ethical Approval

This study enrolled adolescents aged 17–24 years with clinically diagnosed acne vulgaris (AV), recruited via purposive sampling from SMK Jayawisata, East Jakarta, Indonesia. The study protocol was reviewed and approved by the Health Research Ethics Committee of the Faculty of Medicine, Universitas Indonesia—Dr. Cipto Mangunkusumo Hospital (Approval No. KET-505/UN2.FI/ETIK/PPM.00.02/2024, dated 1 April 2024). Written informed consent was obtained from all participants before enrollment. Healthy control data were obtained from a publicly available dataset consisting of 12 Indonesian adolescents matched by age (17–24 years), sex distribution, and Fitzpatrick skin type IV–V. Both acne and control samples were sequenced using the Illumina MiSeq platform targeting the V3–V4 region of the 16S rRNA gene, ensuring compatibility in sequencing chemistry and read structure.

2.2. Clinical Assessment and Sample Collection

Demographic and clinical information, including allergy status, medication use, and family history of AV, was collected through structured interviews. Skin examinations to

confirm the diagnosis of acne vulgaris were conducted by the researcher. Acne severity was evaluated using the Global Acne Grading System (GAGS), which categorizes acne based on lesion type and distribution across anatomical regions. For microbiome sampling, lesion material (including comedones and pustules) from each participant was collected and pooled into a single composite sample prior to DNA extraction to obtain a representative microbial profile for each individual. For each subject, 2–6 lesions were pooled into a sterile tube containing ATL buffer (Qiagen, Hilden, Germany) and stored at −20 °C until DNA extraction.

2.3. DNA Extraction and 16S rRNA Gene Sequencing

Total genomic DNA was extracted using the QIAamp DNA Micro Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. DNA concentration and purity were assessed using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and Qubit Fluorometer (Invitrogen, Carlsbad, CA, USA). The V3–V4 hypervariable region of the bacterial 16S rRNA gene was amplified using primers 341F and 805R. Library preparation was performed using the Nextera XT Index Kit (Illumina, San Diego, CA, USA). Libraries were purified with AMPure XP beads (Beckman Coulter, Brea, CA, USA), quantified, normalized to 4 nM, pooled, and denatured according to Illumina protocols. Sequencing was carried out on the Illumina MiSeq platform.

2.4. Bioinformatics and Statistical Analysis

Negative extraction and PCR controls (Qiagen ATL buffer processed without biological material) were included in each sequencing batch and subjected to the same DNA extraction, PCR amplification, library preparation, and QIIME2 (version 2024.5; https://qiime2.org) processing steps as the study samples. ASVs detected in negative controls above 0.1% relative abundance were considered potential contaminants and removed from the dataset prior to downstream analyses. This filtering step was performed to reduce the impact of low-biomass–associated contaminants commonly observed in skin microbiome studies.

All raw FASTQ files from both the acne and healthy control datasets were imported into a single QIIME2 environment (version 2024.5) to minimize batch-related artifacts [9]. Demultiplexing and quality control were performed uniformly, followed by denoising using Deblur with a fixed trimming length of 220 bp [10]. Representative sequences were aligned with MAFFT (version 7.505; https://mafft.cbrc.jp/alignment/software/ (accessed on 4 June 2025)) [11], masked, and used to construct phylogenetic trees with FastTree [12]. Taxonomic assignment was performed using the Greengenes2 (version 2024.09; Greengenes Project, Boulder, CO, USA) reference classifier, which follows current prokaryotic nomenclature standards (ICSP/IJSEM, LPSN, NCBI Taxonomy). The taxonomic profiles were visualized as barplots. To ensure comparability across samples, rarefaction-based normalization was applied at a depth of 692 reads, corresponding to the minimum sequencing depth in the combined dataset (Figure S1). Although this depth is low, it was necessary to retain all acne lesion samples, reflecting the inherently low biomass of skin microbiome specimens.

Alpha diversity indices (Shannon, Simpson, Chao1, and Faith's phylogenetic diversity) and beta diversity metrics (Bray–Curtis and Jaccard) were calculated to compare microbial diversity between healthy and lesion samples. PERMANOVA tests (999 permutations) were performed using QIIME2's beta-group-significance function to assess group differences in Bray–Curtis and Jaccard dissimilarities, and effect size (R^2) values were extracted from PERMANOVA outputs. Core diversity metrics were visualized using interactive boxplots and principal coordinates analysis (PCoA) plots.

2.5. Predictive Functional Profiling

Functional prediction was performed using PICRUSt2 (version 2.5.0; PICRUSt2 Team, Seattle, WA, USA) [13], which maps 16S rRNA gene sequences to reference genomes to infer KEGG Orthologs (KOs) [14], enzyme commission (EC) numbers, and MetaCyc metabolic pathways [15]. Differential abundance analysis of PICRUSt2-predicted pathways was conducted using DESeq2(version 1.40.0; Bioconductor/R Project, Vienna, Austria) [16] in R. DESeq2's internal normalization and dispersion estimation were applied, and *p*-values were adjusted for multiple testing using the Benjamini–Hochberg false discovery rate (FDR) method to obtain q-values. Log fold-change (logFC) values reported in the Results correspond to DESeq2's shrunken log2 fold-change estimates. Functional profiles were visualized in R using ggplot2 (version 3.5.1; R Project, Vienna, Austria) [17] and pheatmap (version 1.0.10; R Project, Vienna, Austria) [18] to identify microbial functions potentially associated with acne pathophysiology.

2.6. Data Integration and Interpretation

This integrative approach enabled the identification of microbial biomarkers and functional pathways associated with acne lesions, facilitating comparisons between lesional and healthy skin microbiomes as well as between different lesion types.

3. Results

3.1. Sample Characteristics

This study involved 12 adolescent participants (58.4% male, 41.6% female) aged 17–24 years who met the inclusion criteria and provided informed consent. Most respondents had a healthy body mass index (66.7%), with only 16.7% reporting a family history of acne vulgaris (AV). From these participants, a total of 19 acne lesion samples (comedones and pustules) were collected from 7 individuals who provided two lesion samples each. Skin microbiome data from healthy controls (n = 12) sourced from a previous study were used as a comparison group.

3.2. Microbial Composition of Acne Lesions and Healthy Skin

An analysis of 4,733,890 high-quality reads (range 3580–835,219 reads per sample) yielded 1013 OTUs after denoising. Based on demux quality visualization, sequences were trimmed at base position 220 to retain high-quality regions. A sampling depth of 692 reads was selected to maximize inclusion of acne lesion samples in alpha and beta diversity analyses.

Taxonomic classification using the Greengenes2 (2024.09) database revealed distinct microbial profiles between groups. Figure 1 displays the abundance of bacterial phyla. Actinobacteria and Firmicutes dominated both conditions; however, Proteobacteria were enriched in healthy skin, while Firmicutes were more prominent in acne lesions. Several low-abundance phyla, including Planctomycetota, Cyanobacteria, and Campylobacterota, were detected exclusively in healthy skin.

At the genus level (Figure 2, mean ± SD), *Cutibacterium* remained dominant in both groups but was more abundant in healthy skin. Acne lesions showed increased levels of *Staphylococcus* and *Corynebacterium*, along with genera detected exclusively in lesions (*Peptoniphilus*, *Prevotella*, *Dialister*). In contrast, *Paracoccus* was unique to healthy skin. These shifts suggest selective enrichment of inflammation-associated taxa and loss of certain commensals in acne lesions. The detailed taxonomic composition from the phylum to genus level is provided in Supplementary Table S1.

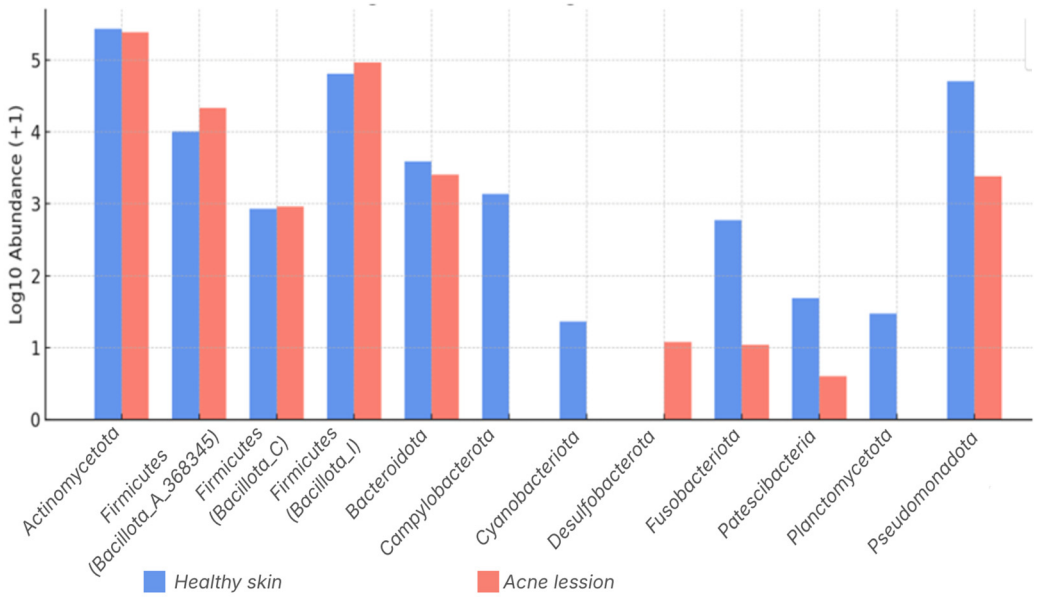

Figure 1. Phylum-level microbial composition in healthy skin and acne lesions. Bars show mean log10-transformed abundance (+1) of each bacterial phylum for healthy skin (blue) and acne lesions (red). Healthy skin: $n = 12$; acne lesions: $n = 19$. Group differences were assessed using the Kruskal–Wallis test with FDR correction; statistically significant results are reported in the main text.

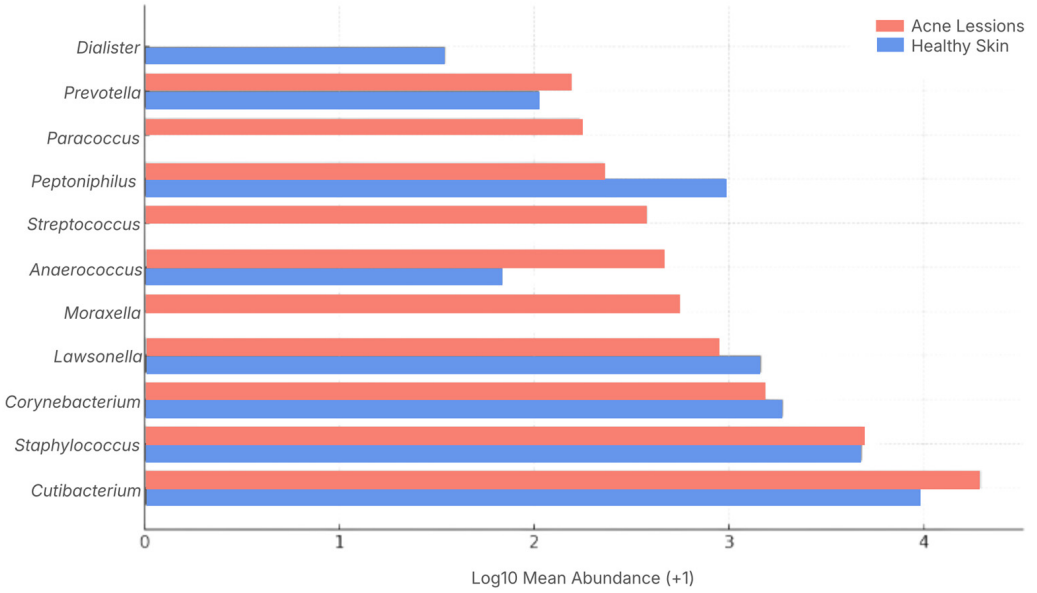

Figure 2. Genus-level microbial composition in healthy skin and acne lesions. Horizontal bars show log10-transformed mean abundance (+1) of the 10 most abundant genera in acne lesions (red) and healthy skin (blue). Healthy skin: $n = 12$; acne lesions: $n = 19$. Statistical comparisons were performed using the Wilcoxon rank–sum test; selected p-values are reported in the main text.

Although the log10-transformed mean abundances appear visually similar between groups, non-parametric tests detected a higher relative abundance of *Staphylococcus* ($p = 0.037$), *Corynebacterium* ($p = 0.042$), and *Peptoniphilus* ($p = 0.048$) in acne lesions compared with healthy skin. These differences in relative abundance are statistically detectable but modest in magnitude and should be interpreted with caution given the sample size and sequencing depth.

3.3. Microbial Diversity Analysis

3.3.1. Alpha Diversity

Alpha diversity analysis was performed using the Shannon index, Simpson evenness, Chao1 richness, and Faith's Phylogenetic Diversity, with results summarized in Figure 3 as boxplots displaying median and interquartile ranges. Overall, healthy skin showed higher microbial diversity values than acne lesions for most indices.

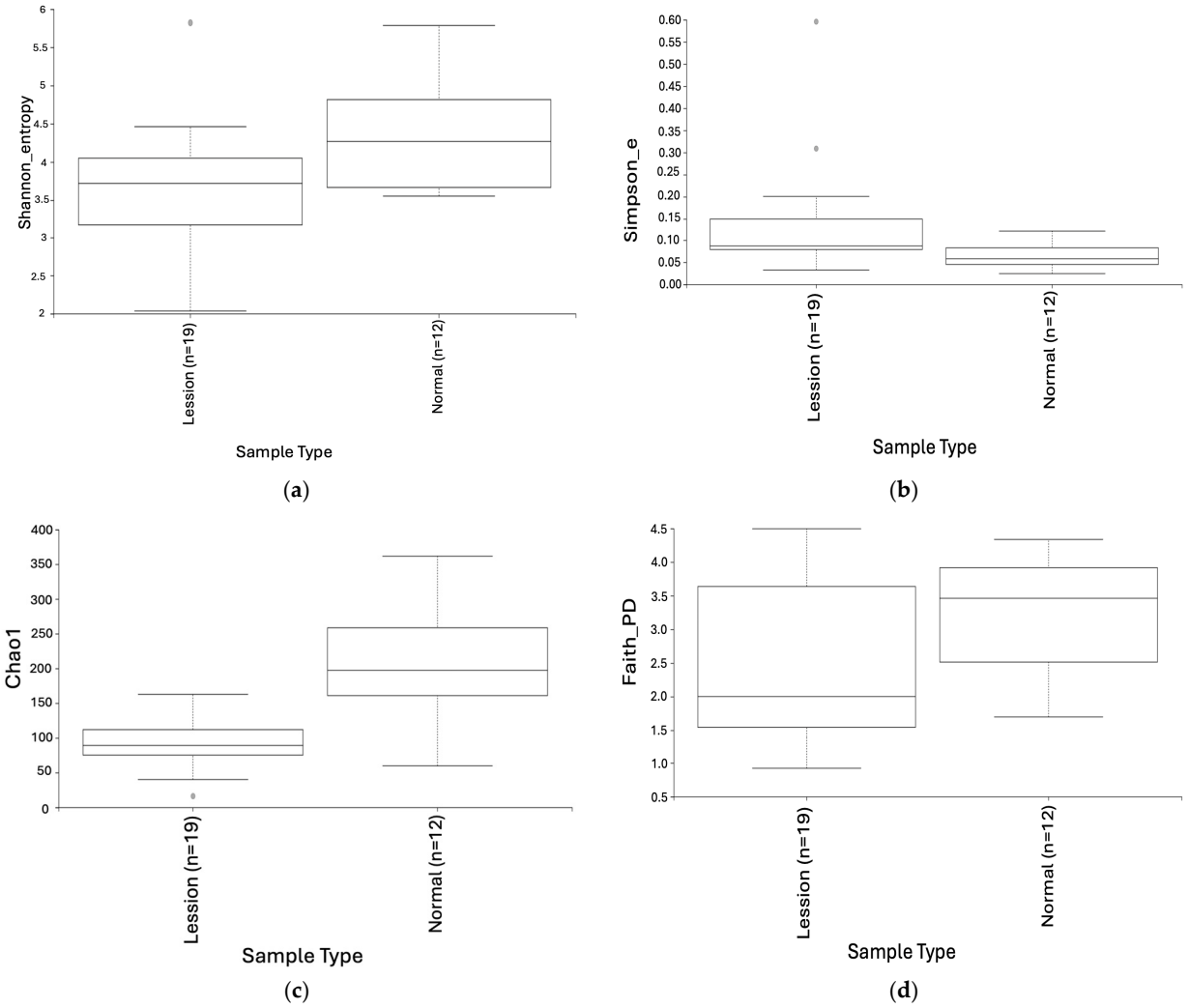

Figure 3. Alpha diversity comparisons between healthy skin and acne lesions. Boxplots display median values and interquartile ranges (IQR) for: (**a**) Shannon diversity index, (**b**) Simpson evenness, (**c**) Chao1 richness, and (**d**) Faith's Phylogenetic Diversity. Healthy skin: n = 12; Acne lesions: n = 19. Rarefied sequencing depth: 692 reads/sample. Statistical differences were evaluated using the Kruskal–Wallis test with FDR correction, with significant results indicated as q < 0.05.

The Shannon index, which captures both richness and evenness, had a higher median and wider distribution in healthy skin compared to lesion samples. However, this difference was not statistically significant (H = 3.79, p = 0.0516, q = 0.0516). The Simpson-e index, reflecting community evenness, was significantly higher in lesion samples (H = 6.32, p = 0.0119, q = 0.0119), suggesting a more uniform taxonomic distribution, potentially due to the dominance of fewer genera in inflamed skin. Chao1 richness was significantly higher in healthy skin (H = 15.16, p = 0.000099, q = 0.000099), indicating that healthy skin harbors a broader array of microbial taxa. Faith's Phylogenetic Diversity was also higher in healthy skin, although this difference did not reach statistical significance (H = 3.04, p = 0.0812, q = 0.0812).

Collectively, these findings indicate that acne lesions exhibit reduced richness and phylogenetic diversity relative to healthy skin, whereas evenness appears higher in lesion sites. This pattern is consistent with microbial disruption in inflammatory skin conditions.

3.3.2. Beta Diversity

Beta diversity analyses further demonstrated marked differences in overall microbial community structure between acne lesions and healthy skin. As shown in Figure 4, Principal Coordinates Analysis (PCoA) based on Bray–Curtis dissimilarity revealed distinct separation between groups, with healthy skin samples clustering tightly and lesion samples displaying broader dispersion. This pattern suggests that healthy skin maintains a relatively stable and uniform microbial structure, whereas acne lesions are characterized by greater inter-individual variability and community disruption. A similar separation was observed using the Jaccard distance, which considers only the presence or absence of taxa.

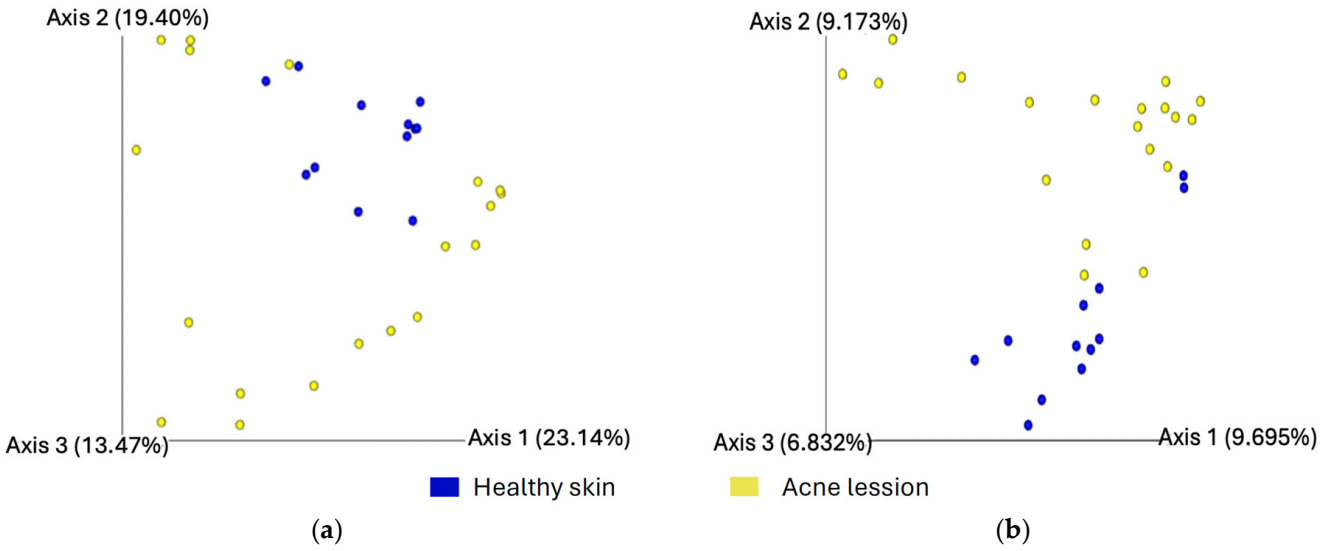

Figure 4. Beta diversity analysis of healthy skin and acne lesions. Principal Coordinates Analysis (PCoA) based on: (**a**) Bray–Curtis dissimilarity (abundance-based), and (**b**) Jaccard distance (presence/absence-based). Healthy skin: $n = 12$; Acne lesions: $n = 19$. Group differences were tested using PERMANOVA, with significant p-values ($p < 0.05$) indicating distinct microbial community structures.

PERMANOVA confirmed significant differences between acne and healthy skin communities, with effect sizes of $R^2 = 0.142$ ($p = 0.004$) for Bray–Curtis (Figure S2A) and $R^2 = 0.168$ ($p = 0.001$) for Jaccard dissimilarities (Figure S2B). Importantly, samples did not cluster according to dataset origin or sequencing batch, indicating minimal batch effect after uniform preprocessing and rarefaction. These findings suggest that acne vulgaris is not only associated with changes in microbial diversity but also with substantial compositional restructuring of the skin microbiome. Such shifts may contribute to the pathogenesis of acne through dysbiosis and altered host–microbe interactions.

3.4. Microbial Functional Analysis

Metabolic function prediction using PICRUSt2 revealed distinct differences in pathway profiles between acne lesion samples and healthy skin. The separation observed between sample types suggests functional divergence in microbial communities associated with acne pathology. A total of 391 metabolic pathways were predicted across all samples, including both lesion and healthy skin groups. For clarity, only the top 30 most abundant pathways are visualized in Figure 5 Most pathways in the heatmap displayed higher abundance

(indicated in red) in healthy skin samples compared to lesion samples. These findings indicate a shift in the metabolic potential of the skin microbiota related to the pathological condition of acne vulgaris.

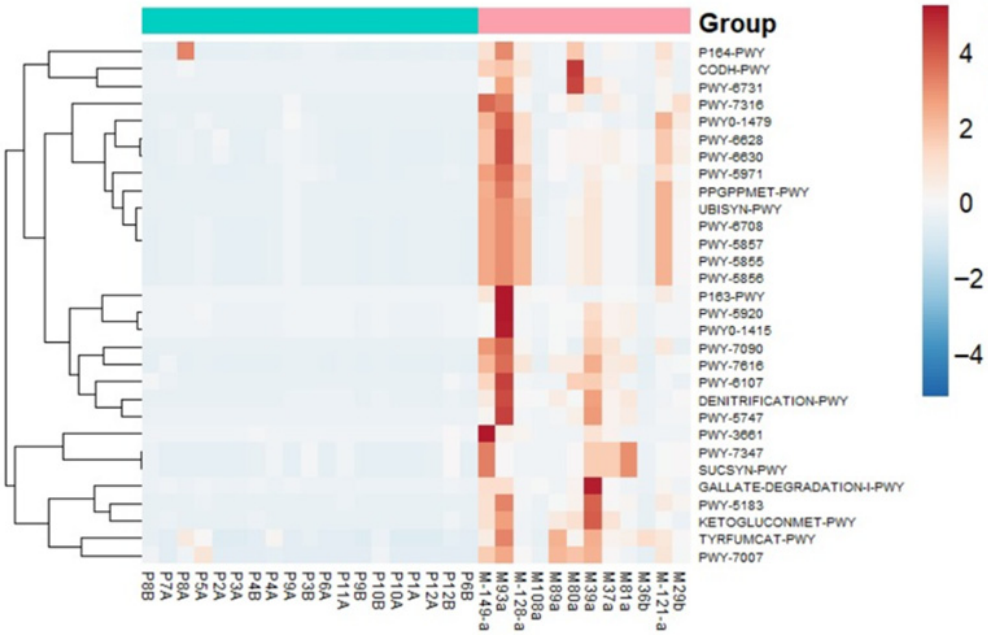

Figure 5. Functional pathway profiles predicted from PICRUSt2. Heatmap shows the top 30 most abundant MetaCyc pathways in healthy skin (pink) and acne lesions (turquoise). Color intensity reflects normalized pathway abundance. Healthy skin: $n = 12$; Acne lesions: $n = 19$. Differential pathway abundance was assessed using Kruskal–Wallis with FDR correction, with significantly enriched pathways defined as FDR < 0.05, $p < 0.05$, and $|\log FC| > 1$.

Out of the 391 analyzed pathways, 163 showed significant differences between acne lesions and healthy skin based on strict statistical and biological criteria (FDR < 0.05, p-value < 0.05, and $|\log FC| > 1$). Only 3 pathways were found to be more abundant in lesion samples, while the remaining 160 pathways were more enriched in the healthy skin group. To assess the reliability of functional inference, NSTI (Nearest Sequenced Taxon Index) values were calculated for all samples. The NSTI counts are provided in Supplementary Table S2.

Functional analysis of microbial enzymatic activity related to acne pathogenesis focused on two key enzymes: lipase (EC:3.1.1.3) and hyaluronate lyase (EC:4.2.2.1). Functional predictions based on 16S rRNA data revealed differences in the relative abundance of these enzymes between healthy skin and acne lesions. Lipase was found to be more abundant in acne lesion samples, whereas hyaluronate lyase showed higher abundance in healthy skin.

4. Discussion

This study presents an integrative analysis of the skin microbiome in adolescents with acne vulgaris (AV), highlighting significant differences in taxonomic composition, microbial diversity, and functional potential compared to healthy controls. The findings align with growing evidence that microbial dysbiosis is a central factor in the pathogenesis of acne, influencing both host immune responses and local skin physiology [3,19].

At the broader taxonomic level, both acne-affected and healthy skin were dominated by members of the phylum *Actinobacteria*. However, the proportion of these bacteria appeared lower in acne lesions. Interestingly, *Cutibacterium* was found more abundantly in healthy skin, while lesion samples showed a higher prevalence of genera like *Staphylococcus*,

Corynebacterium, and *Peptoniphilus*. This shift in microbial profiles is consistent with prior research, which suggests that certain *C. acnes* strains, particularly the IA1 phylotype, may promote inflammation, while others could have a neutral or even protective role [20,21]. The emergence of *Peptoniphilus* in acne lesions might reflect compromised skin integrity, allowing less common or opportunistic microbes to colonize.

Beyond these dominant taxa, several phyla and genera were detected exclusively in one group. Planctomycetota, Cyanobacteria, and Campylobacterota were found only in healthy skin, suggesting the loss of low-abundance commensals that may normally contribute to ecological stability. In contrast, taxa such as *Prevotella* and *Dialister* appeared only in acne lesions, consistent with their tendency to thrive in anaerobic or inflamed microenvironments. Their selective presence indicates that acne-associated dysbiosis involves not only shifts in major genera but also the enrichment of organisms that may benefit from the altered follicular milieu during inflammation.

Although additional phylum- and genus-level differences were observed, many of these variations were small and did not reach statistical significance. These minor changes are unlikely to have biological relevance, and the revised manuscript clarifies which findings are supported by statistical analysis to prevent overinterpretation.

One notable observation is the comparable overall abundance of *Cutibacterium* in both healthy and acne-affected skin. While *C. acnes* has long been implicated in acne, the recent literature highlights that total abundance is less informative than strain-level diversity. Pro-inflammatory strains, such as those belonging to phylotype IA1, are often enriched in acne, whereas type II strains are more commonly found in healthy skin. Because 16S rRNA gene sequencing cannot resolve taxa beyond the genus level, our results likely mask the underlying strain-level differences. Studies by Fitz-Gibbon et al. [21] and Dagnelie et al. [22] support this, showing that acne severity is driven by specific phylotypes rather than by an overall increase in *C. acnes*. This methodological limitation is now acknowledged to provide more accurate context for our findings.

When examining microbial richness, alpha diversity analyses revealed that healthy skin harbored more diverse and phylogenetically rich microbial communities. This diversity is often associated with a resilient, balanced skin microbiome [23]. In contrast, acne lesions displayed lower richness but greater evenness, suggesting that only a few microbial taxa dominate. Such patterns are frequently observed in inflamed environments, where microbial selection pressures such as altered sebum levels or oxidative stress favor the survival of specific bacteria over others [24]. Reduced diversity, as shown in previous studies, may impair immune balance and worsen inflammation in acne [25–27].

Beta diversity, analyzed through Bray–Curtis and Jaccard distance metrics, showed a clear separation between the acne and healthy skin groups in PCoA plots. Healthy skin samples tended to cluster closely together, suggesting a relatively uniform microbial profile among individuals without acne. Conversely, lesion samples were widely scattered, indicating greater variability and instability in microbial community structure. A similar observation was reported by a previous study which also found that acne lesions exhibited high inter-sample diversity, suggesting a disrupted and less stable microbiome compared to healthy skin [6,27]. These compositional differences were statistically significant, as confirmed by PERMANOVA ($p < 0.05$).

These microbial differences may also reflect ecological pressures associated with tropical environmental conditions. High humidity and consistently warm temperatures increase skin hydration and stimulate sebum production, providing a nutrient-rich environment that selectively favors lipophilic and moisture-tolerant microbes [4,8]. These factors may help explain the reduced richness and altered taxonomic profiles observed in acne lesions, where elevated sebum levels and follicular occlusion create conditions conducive

to the overgrowth of specific taxa while suppressing others [3]. Additionally, variations in perspiration levels, air pollution, and local skincare habits common in tropical climates may further modulate microbial community structure [5]. Taken together, these environmental influences highlight the importance of examining acne-related dysbiosis within region-specific ecological contexts.

Acne vulgaris typically begins with a non-inflammatory phase characterized by the formation of early lesions due to microbial colonization, particularly by *Cutibacterium acnes*. During this stage, microbial load in hair follicles tends to be high, likely due to the rapid proliferation of *C. acnes* in the occluded follicular environment. This microbial overgrowth eventually triggers an immune response, marking the transition to the inflammatory phase, indicated by the appearance of papules and pustules. In this phase, immune cells such as neutrophils actively eliminate microorganisms through phagocytosis and the release of inflammatory mediators. As a result, microbial abundance decreases in inflamed lesions, suggesting that microbial communities are more abundant in non-inflammatory lesions than in inflammatory ones due to increased host immune activity [28].

Heatmap visualization reveals distinct patterns of metabolic pathway distribution between groups. These differences suggest potential biological relevance, possibly reflecting more complex microbial interactions or specific pathways involved in lesion development. Although predictive in nature, these findings provide an initial perspective on the functional shifts in the skin microbiome associated with acne [28]. The results highlight the importance of applying integrative multi-omics approaches, such as metatranscriptomics or metabolomics, for a deeper exploration of microbial roles in acne pathogenesis [29,30].

Skin affected by acne lesions showed fewer and less diverse metabolic pathways compared to healthy skin. This likely reflects a loss of important microbial functions or a general drop in microbial metabolic activity in acne-prone areas. On the other hand, healthy skin had a richer and more diverse set of pathways, suggesting a more balanced and stable microbiome. These functional differences may help explain why healthy skin is better at maintaining barrier integrity and resisting inflammation [31].

The enrichment of pyrimidine biosynthesis pathways may reflect increased nucleotide turnover or microbial activity associated with inflamed skin microenvironments. This pathway is essential for immune cell proliferation and has been linked to inflammation; in fact, its inhibition has been used in treating autoimmune conditions like rheumatoid arthritis. The presence of this pathway may reflect the inflammatory nature of acne lesions [32]. Additionally, we observed a higher abundance of isoprenoid biosynthesis pathways in the lesions. These compounds help bacteria stabilize their membranes in harsh environments by producing hopanoids, which act like sterols. This likely represents a microbial response to stressors such as oxidative damage, pH changes, and osmotic pressure within acne lesions. Together, these findings highlight how microbial functions may shift in response to the inflammatory environment of acne-affected skin [33].

One of the pathways more abundant in healthy skin, PWY-7090, is involved in the biosynthesis of UDP-2,3-diacetamido-2,3-dideoxy-α-D-mannuronate, a nucleotide-sugar component of bacterial surface polysaccharides such as O-antigens in lipopolysaccharides (LPS) of Gram-negative bacteria. This pathway plays a critical role in mediating microbial-host immune interactions. Its presence in the healthy skin microbiota suggests a contribution from commensal Gram-negative bacteria in maintaining an immunologically balanced and tolerant skin environment. The activity of PWY-7090 may help sustain microbial homeostasis and support the skin's natural defense mechanisms against pathogen colonization and excessive inflammation [34].

Moreover, the diverse metabolic pathways identified in healthy skin samples suggest a balanced biosynthetic and degradative activity, including fatty acid metabolism, vitamin

biosynthesis, and denitrification. This diversity supports a competitive yet complementary microbial community that contributes to the integrity and physiological function of healthy skin [31].

Cutibacterium acnes is known to produce various enzymes, including lipases, proteases, phosphatases, hyaluronate lyases, and neuraminidases, which can directly damage the hair follicle structure, sebaceous glands, and extracellular matrix. These enzymatic activities exacerbate local inflammation and accelerate lesion progression. Specifically, free fatty acids produced by lipase activity are implicated in comedogenesis [6]. Functional analysis of lipase and hyaluronate lyase predicted a slightly higher abundance of lipase in acne lesions than in healthy skin. This aligns with previous studies linking increased *C. acnes* lipase activity to acne severity. Lipase contributes to the hydrolysis of sebum triglycerides into free fatty acids, which can induce irritation, disrupt skin barrier integrity, and activate inflammatory responses in the pilosebaceous unit. Previous research reported that *C. acnes* strains associated with severe acne exhibit higher lipolytic activity compared to strains from mild to moderate cases. Elevated lipase activity may result in increased free fatty acid levels, known to promote comedone formation and inflammation [35,36].

Hyaluronate lyase is another enzyme involved in acne pathogenesis. In this study, hyaluronate lyase was predicted to be more abundant in healthy skin than in lesion samples. Recent studies have identified two major variants of hyaluronate lyase in *C. acnes*: HylA and HylB [37,38]. HylA produces large hyaluronic acid fragments that trigger strong inflammation, whereas HylB generates smaller, anti-inflammatory fragments. In healthy skin, hyaluronidase is still expressed but exhibits different activity and effects. The HylB variant is more prevalent in *C. acnes* strains from healthy skin, playing a role in maintaining homeostasis and reducing inflammatory potential [37].

This study has several limitations that should be acknowledged. The relatively small number of participants reduces statistical power and limits the generalizability of the findings. Although the observed microbial differences provide preliminary insight into dysbiosis associated with acne lesions, these results should be interpreted as exploratory. Larger cohorts with broader demographic representation, as well as longitudinal sampling to capture temporal variation, will be necessary to validate these microbial patterns and strengthen the conclusions.

The use of an external dataset for healthy controls introduces the potential for batch effects, despite uniform preprocessing and the use of comparable sequencing platforms targeting the same 16S rRNA region. Technical variation therefore cannot be completely ruled out. Additionally, the rarefaction depth of 692 reads—selected to retain all samples—may reduce sensitivity for detecting low-abundance taxa.

Although negative extraction and PCR controls were included and ASVs detected above 0.1% relative abundance were removed, the possibility of residual low-biomass contaminants (e.g., *Ralstonia*, *Sphingomonas*) cannot be fully excluded. Furthermore, because functional inferences were generated from 16S rRNA gene profiles using PICRUSt2, these pathway predictions are indirect and should not be interpreted as equivalent to whole-metagenome functional measurements. The 16S approach also lacks strain-level resolution, preventing assessment of *Cutibacterium acnes* phylotypes that differ in pathogenic potential.

These limitations underscore the need for larger, prospectively collected, deeply sequenced, and strain-resolved metagenomic datasets in future investigations. Taken together, the data suggest that acne-affected skin is marked not only by shifts in microbial composition but also by potential alterations in microbial functional capacity. This functional imbalance complements the taxonomic changes observed and supports the hypothesis that the skin microbiome may contribute to acne not merely through its composition but

through its metabolic activity, including the production of bioactive compounds that may influence local inflammation and host skin physiology [20].

5. Conclusions

This study highlights that adolescents with acne vulgaris experience notable changes not only in the types of microbes present on their skin but also in the biological activities those microbes perform. Compared to individuals with healthy skin, acne lesions show a decline in microbial diversity and a higher abundance of certain bacteria like *Staphylococcus* and *Peptoniphilus*. Microbial functional predictions revealed significant differences in metabolic pathway profiles between healthy skin and acne lesions. The majority of pathways enriched in healthy skin were associated with higher metabolic capacity, reflecting a more stable and potentially protective microbial functionality. Furthermore, enzymatic activities, particularly lipase and hyaluronate lyase, play pivotal roles in acne pathogenesis. These enzymes demonstrated differential abundance patterns between healthy and lesional skin, underscoring their functional relevance in follicular colonization, barrier disruption, and inflammatory responses. These findings suggest potential alterations in the predicted metabolic potential of the skin microbiome in acne lesions; however, confirmation using transcriptomic, proteomic, or metabolomic approaches would be necessary to validate these predictions.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/biomedinformatics6010001/s1, Figure S1: Rarefaction curves for 16S rRNA sequencing data.; Table S1: Taxonomic composition of the skin microbiome from the phylum to genus level.; Figure S2: Permanova analysis for beta diversity; Table S2: SNSTI count for all samples.

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Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to privacy and ethical restrictions related to human subject research.

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Abbreviations

The following abbreviations are used in this manuscript:

16S-rRNA	16S Ribosomal Ribonucleic Acid
OTU	Operational Taxonomic Units
AV	Acne vulgaris
SMK	Sekolah Menengah Kejuruan
DNA	Deoxyribonucleic Acid
QIIME2	Quantitative Insights into Microbial
MAFFT	Multiple Alignment using Fast Fourier Transform
NSTI	Nearest Sequenced Taxon Index
PCoA	Principal Coordinates Analysis
PICRUSt	Phylogenetic Investigation of Communities by Reconstruction of Unobserved States
KEGG	Kyoto Encyclopedia of Genes and Genomes
KO	KEGG ortholog
EC	Enzyme Commission (Number)
BMI	Body Mass Index
PERMANOVA	Permutational Multivariate Analysis of Variance

References

1. Shamloul, G.; Khachemoune, A. An updated review of the sebaceous gland and its role in health and diseases Part 1: Embryology, evolution, structure, and function of sebaceous glands. *Dermatol. Ther.* **2021**, *34*, e14695. [CrossRef]
2. Akaza, N.; Akamatsu, H.; Numata, S.; Yamada, S.; Yagami, A.; Nakata, S.; Matsunaga, K. Microorganisms inhabiting follicular contents of facial acne are not only *Propionibacterium* but also *Malassezia* spp. *J. Dermatol.* **2016**, *43*, 906–911.
3. Dréno, B.; Dagnelie, M.A.; Khammari, A.; Corvec, S. The Skin Microbiome: A New Actor in Inflammatory Acne. *Am. J. Clin. Dermatol.* **2020**, *21*, 18–24.
4. Asrianti, N.I.; Setyaningrum, T.; Setiawati, Y.; Widia, Y. Factors that Influence The Onset of Acne Vulgaris: Retrospective Study. *Berk. Ilmu Kesehat. Kulit Dan. Kelamin* **2024**, *36*, 98–103. [CrossRef]
5. Smythe, P.; Wilkinson, H.N. The Skin Microbiome: Current Landscape and Future Opportunities. *Int. J. Mol. Sci.* **2023**, *24*, 3950. [CrossRef] [PubMed]
6. Akaza, N.; Takasaki, K.; Nishiyama, E.; Usui, A.; Miura, S.; Yokoi, A.; Futamura, K.; Suzuki, K.; Yashiro, Y.; Yagami, A. The Microbiome in Comedonal Contents of Inflammatory Acne Vulgaris is Composed of an Overgrowth of *Cutibacterium* Spp. and Other Cutaneous Microorganisms. *Clin. Cosmet. Investig. Dermatol.* **2022**, *15*, 2003–2012.
7. Meisel, J.S.; Hannigan, G.D.; Tyldsley, A.S.; Sanmiguel, A.J.; Hodkinson, B.P.; Zheng, Q.; Grice, E.A. Skin microbiome surveys are strongly influenced by experimental design. *J. Investig. Dermatol.* **2016**, *136*, 947–956.
8. Skowron, K.; Bauza-Kaszewska, J.; Kraszewska, Z.; Wiktorczyk-Kapischke, N.; Grudlewska-Buda, K.; Kwiecińska-Piróg, J.; Wałecka-Zacharska, E.; Radtke, L.; Gospodarek-Komkowska, E. Human skin microbiome: Impact of intrinsic and extrinsic factors on skin microbiota. *Microorganisms* **2021**, *9*, 543. [CrossRef]
9. Bolyen, E.; Rideout, J.R.; Dillon, M.R.; Bokulich, N.A.; Abnet, C.C.; Al-Ghalith, G.A.; Alexander, H.; Alm, E.J.; Arumugam, M.; Asnicar, F.; et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat. Biotechnol.* **2019**, *37*, 852–857. [CrossRef] [PubMed]
10. Amir, A.; McDonald, D.; Navas-Molina, J.A.; Kopylova, E.; Morton, J.T.; Zech Xu, Z.; Kightley, E.P.; Thompson, L.R.; Hyde, E.R.; Gonzalez, A.; et al. Deblur Rapidly Resolves Single-Nucleotide Community Sequence Patterns. *MSystems* **2017**, *2*, e00191-16. [PubMed]
11. Katoh, K.; Standley, D.M. MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Mol. Biol. Evol.* **2013**, *30*, 772–780. [CrossRef]
12. Price, M.N.; Dehal, P.S.; Arkin, A.P. Fasttree: Computing large minimum evolution trees with profiles instead of a distance matrix. *Mol. Biol. Evol.* **2009**, *26*, 1641–1650. [CrossRef] [PubMed]
13. Douglas, G.M.; Maffei, V.J.; Zaneveld, J.; Yurgel, S.N.; Brown, J.R.; Taylor, C.M.; Huttenhower, C.; Langille, M.G.I. PICRUSt2: An improved and customizable approach for metagenome inference. *bioRxiv* **2019**, *preprint*. [CrossRef]
14. Kanehisa, M.; Goto, S.; Sato, Y.; Furumichi, M.; Tanabe, M. KEGG for integration and interpretation of large-scale molecular data sets. *Nucleic Acids Res.* **2012**, *40*, D109–D114. [CrossRef]
15. Caspi, R.; Billington, R.; Keseler, I.M.; Kothari, A.; Krummenacker, M.; Midford, P.E.; Ong, W.K.; Paley, S.; Subhraveti, P.; Karp, P.D. The MetaCyc database of metabolic pathways and enzymes-a 2019 update. *Nucleic Acids Res.* **2020**, *48*, D445–D453. [CrossRef]

16. Love, M.I.; Huber, W.; Anders, S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* **2014**, *15*, 550. [CrossRef]
17. Gómez-Rubio, V. ggplot2—Elegant Graphics for Data Analysis (2nd Edition). *J. Stat. Softw.* **2017**, *77*, 1–3. [CrossRef]
18. Kolde, R. Pheatmap: Pretty Heatmaps. R Package Version 1.0.10. 2012. Available online: https://CRAN.R-project.org/package=pheatmap (accessed on 4 June 2025).
19. Salem, I.; Ramser, A.; Isham, N.; Ghannoum, M.A. The gut microbiome as a major regulator of the Gut-Skin Axis. *Front. Microbiol.* **2018**, *9*, 1459. [CrossRef]
20. Barnard, E.; Shi, B.; Kang, D.; Craft, N.; Li, H. The balance of metagenomic elements shapes the skin microbiome in acne and health. *Sci. Rep.* **2016**, *6*, 39491. [CrossRef] [PubMed]
21. Fitz-Gibbon, S.; Tomida, S.; Chiu, B.H.; Nguyen, L.; Du, C.; Liu, M.; Elashoff, D.; Erfe, M.C.; Loncaric, A.; Kim, J.; et al. Propionibacterium acnes strain populations in the human skin microbiome associated with acne. *J. Investig. Dermatol.* **2013**, *133*, 2152–2160. [CrossRef] [PubMed]
22. Dagnelie, M.A.; Corvec, S.; Saint-Jean, M.; Nguyen, J.M.; Khammari, A.; Dréno, B. Cutibacterium acnes phylotypes diversity loss: A trigger for skin inflammatory process. *J. Eur. Acad. Dermatol. Venereol.* **2019**, *33*, 2340–2348. [CrossRef]
23. Byrd, A.L.; Belkaid, Y.; Segre, J.A. The human skin microbiome. *Nat. Rev. Microbiol.* **2018**, *16*, 143–155. [CrossRef] [PubMed]
24. Cavallo, I.; Sivori, F.; Truglio, M.; De Maio, F.; Lucantoni, F.; Cardinali, G.; Pontone, M.; Bernardi, T.; Sanguinetti, M.; Capitanio, B.; et al. Skin dysbiosis and Cutibacterium acnes biofilm in inflammatory acne lesions of adolescents. *Sci. Rep.* **2022**, *12*, 21104. [CrossRef]
25. Du, Y.; Li, B.; Yang, J.; Zhang, Y.; Qi, F.; Meng, H. Analysis of Skin Microbiome in Facial and Back Acne Patients Based on High-Throughput Sequencing. *J. Cosmet. Dermatol.* **2025**, *24*, e70259. [CrossRef] [PubMed]
26. Podwojniak, A.; Tan, I.J.; Sauer, J.; Neubauer, Z.; Rothenberg, H.; Ghani, H.; Parikh, A.K.; Cohen, B. Acne and the cutaneous microbiome: A systematic review of mechanisms and implications for treatments. *J. Eur. Acad. Dermatol. Venereol.* **2025**, *39*, 793–805. [CrossRef]
27. Schneider, A.M.; Nolan, Z.T.; Banerjee, K.; Paine, A.R.; Cong, Z.; Gettle, S.L.; Longenecker, A.L.; Zhan, X.; Agak, G.W.; Nelson, A.M. Evolution of the facial skin microbiome during puberty in normal and acne skin. *J. Eur. Acad. Dermatol. Venereol.* **2023**, *37*, 166–175. [CrossRef]
28. Mayslich, C.; Grange, P.A.; Dupin, N. Cutibacterium acnes as an Opportunistic Pathogen: An Update of Its Virulence-Associated Factors. *Microorganisms* **2021**, *9*, 303. [CrossRef]
29. Kang, D.; Shi, B.; Erfe, M.C.; Craft, N.; Li, H. Vitamin B12 modulates the transcriptome of the skin microbiota in acne pathogenesis. *Sci. Transl. Med.* **2015**, *7*, 293ra103. [CrossRef] [PubMed]
30. Wang, X.; Wu, Y.; Zhao, P.; Wang, X.; Wu, W.; Yang, J. The causal relationship between serum metabolites and acne vulgaris: A Mendelian randomization study. *Sci. Rep.* **2024**, *14*, 11045. [CrossRef]
31. Oh, J.; Byrd, A.L.; Park, M.; NISC Comparative Sequencing Program; Kong, H.H.; Segre, J.A. Temporal Stability of the Human Skin Microbiome. *Cell* **2016**, *165*, 854–866. [CrossRef]
32. Scherer, S.; Oberle, S.G.; Kanev, K.; Gerullis, A.-K.; Wu, M.; de Almeida, G.P.; Puleston, D.J.; Baixauli, F.; Aly, L.; Greco, A.; et al. Pyrimidine de novo synthesis inhibition selectively blocks effector but not memory T cell development. *Nat. Immunol.* **2023**, *24*, 501–515. [CrossRef]
33. Kuntz, L.; Tritsch, D.; Grosdemange-Billiard, C.; Hemmerlin, A.; Willem, A.; Bach, T.J.; Rohmer, M. Isoprenoid biosynthesis as a target for antibacterial and antiparasitic drugs: Phosphonohydroxamic acids as inhibitors of deoxyxylulose phosphate reducto-isomerase. *Biochem. J.* **2005**, *386*, 127–135. [CrossRef]
34. Atwal, S.; Chuenklin, S.; Bonder, E.M.; Flores, J.; Gillespie, J.J.; Driscoll, T.P.; Salje, J. Discovery of a diverse set of bacteria that build their cell walls without the canonical peptidoglycan polymerase apbp. *mBio* **2021**, *12*, e0134221. [CrossRef]
35. Bashar, A.M.; Najem, W.S.; Alrifai, S.B.; Mukhlif, E.D. Assessment of lipase activity for Cutibacterium acnes in relation to acne vulgaris disease severity in a group of Iraqi patients. *IOSR J. Dent. Med. Sci. (IOSR-JDMS) E-ISSN* **2020**, *19*, 57–61.
36. Burkhart, C.G. Assessment of Cutibacterium acnes: Acne Biofilm, Comedones, and Future Treatments for Acne. *Open Dermatol. J.* **2024**, *18*, e18743722279314. [CrossRef]
37. Hajam, I.A.; Katiki, M.; McNally, R.; Lázaro-Díez, M.; Kolar, S.; Chatterjee, A.; Gonzalez, C.; Paulchakrabarti, M.; Choudhury, B.; Caldera, J.R.; et al. Functional divergence of a bacterial enzyme promotes healthy or acneic skin. *Nat. Commun.* **2023**, *14*, 8061. [CrossRef]
38. Nazipi, S.; Stødkilde-Jørgensen, K.; Scavenius, C.; Brüggemann, H. The skin bacterium propionibacterium acnes employs two variants of hyaluronate lyase with distinct properties. *Microorganisms* **2017**, *5*, 57. [CrossRef] [PubMed]