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# Shotgun metagenomic profiling of bacterial communities from dry axillary skin swabs

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## Abstract

# Background

Shotgun metagenomic sequencing can provide taxonomic resolution beyond that of targeted 16S rRNA gene sequencing. However, its application to skin swabs is challenging because these samples often contain low amounts of microbial DNA and a high proportion of host DNA.

# Objective

To explore whether dry skin swabs can be used for shotgun metagenomic studies of the skin microbiome.

# Methods

Dry swab samples were collected from the left axilla of eight healthy volunteers at baseline and after 7 and 14 days. Total DNA was extracted and processed using a modified low-input library-preparation workflow, followed by paired-end shotgun metagenomic sequencing. Reads classified as human were excluded before taxonomic profiling with Kraken2 and Bracken. A microbial community DNA standard was included at the library-preparation stage to assess sequencing and taxonomic classification.

# Results

DNA concentrations in skin swab extracts were below the detection limit of fluorometric quantification. Sequencing revealed a variable proportion of reads classified as human across samples. After exclusion of human reads and normalization of bacterial taxonomic counts, profiles appeared more similar within individuals than between individuals over the two-week sampling period. The workflow produced sequence libraries from low-input skin swab samples without a host DNA depletion step, although the findings remain exploratory.

# Conclusion

Despite very low DNA yields, dry axillary skin swabs generated sequence libraries suitable for exploratory shotgun metagenomic profiling without host DNA depletion. The resulting bacterial community profiles appeared stable within individuals over a two-week period. These findings suggest that dry skin sites may be amenable to shotgun metagenomic analysis, although larger studies incorporating multiple negative controls and additional skin sites are needed.

## Introduction

The skin is home to a distinctive symbiotic collection of microbes. Skin-associated microbes may contribute to epithelial homeostasis, immune education, and defence against potential pathogens (Bay et al., 2020; Grice et al., 2008). These microbes also activate and train skin and immune cells to enhance immune defence and protect against harmful pathogens (Schommer and Gallo, 2013). The physical characteristics of the skin varies across body sites, ranging from oily/sebaceous to moist or dry. This results in topologically diverse microenvironments with unique microbial populations (Byrd et al., 2018; Grice et al., 2008). There are core skin microbial members, but for every individual, the microbial composition is unique (Oh et al., 2014; Richardson et al., 2019). A balanced community of skin-commensal bacteria is essential for maintaining healthy skin. An altered commensal composition or dysbiosis is often associated with inflammatory skin diseases such as acne, atopic dermatitis, and psoriasis (Akaza et al., 2016; Alekseyenko et al., 2013; Nowicka et al., 2022; Weyrich et al., 2015).

Enhanced understanding of the human microbiome and its relationship with the immune system can improve our knowledge of various diseases and lead to new ways to treat them.

Traditionally, culture-based methods are used to identify bacteria in a clinical setting. These methods severely underestimate the diversity of skin bacteria (Melendez et al., 2010; Rhoads et al., 2012a, b). This underestimation is caused primarily by (a) the lack of growth of skin bacteria on laboratory media (Kong and Segre, 2012; Rhoads et al., 2012a), and (b) a low sample size obtained by various skin-bacterial sampling methods (Bjerre et al., 2019; Grice et al., 2008; Kong et al., 2017; Ogai et al., 2018). Culture-independent sequencing approaches, including 16S rRNA gene amplicon sequencing and shotgun metagenomic sequencing, have been used to characterize bacteria that are difficult to culture (Jo et al., 2021; Oh et al., 2014; Rhoads et al., 2012a; Ruuskanen et al., 2022; Timm et al., 2020; Zeeuwen et al., 2012). Shotgun metagenomic sequencing can provide improved taxonomic resolution and access to microbial gene content compared with 16S rRNA gene amplicon sequencing. However, its performance depends on sequencing depth, database quality, and the proportion of host DNA in the sample (Brumfield et al., 2020; Johnson et al., 2019; Ranjan et al., 2016). The low microbial biomass obtained from skin swab sampling may yield DNA amounts that are near or below the detection limit of routine quantification methods (Bjerre et al., 2019; Ogai et al., 2018). In this study, we present a modified methodological approach for the detection of microbial communities from skin dry swabs. DNA was obtained from dry skin swabs and subsequently characterized by shotgun metagenomic sequencing.

## Materials and Methods

The workflow of the experimental design, from sample collection to metagenomic analysis, is depicted in Fig. 1.

# Dry swab sampling from axillary skin

Dry swab samples (hereafter referred to as skin dry swabs [SDS]) were collected from eight healthy volunteers (five women and three men; age range, 24–29 years) for shotgun metagenomic analysis of axillary bacterial diversity. Sample IDs are presented in Table 1. Samples were collected on days 0, 7, and 14. Two scheduled samples were not obtained and are indicated as missing in Table 1. Participants had not received systemic antibiotics during the month preceding sampling. Participants were instructed to shower the evening before but no later than 12 hours before sampling. Furthermore, participants were instructed not to use deodorant after having a shower prior to sample collection. To improve consistency of sampling, all bacterial samples were collected by the same trained investigator by gentle rubbing and rolling for 10 seconds in a 3x3 cm area in the centre of the left armpit using a sterile dry-fibre swab (C1053; Zymo Research). Sampling was performed at baseline and at 7 and 14 days. Swabs with skin bacterial samples were kept in collection tubes prefilled with 1 ml of DNA/RNA shield lysis solution at 4°C until DNA isolation (R1106; Zymo Research). In a separate pilot, three healthy volunteers (all female) aged between 22 – 32 years, axillary skin sampling was performed at one time point with a dry swab as described above from the right axilla and with a wet swab moistened with sterile saline in the left axilla.

Table 1
Participant characteristics, sample identifiers, sampling time points, and sequencing metrics.

| Individuals | Gender, Age (y) | Sample id | | |
|---|---|---|---|---|
| | | Sampling day 0 | Sampling day 7 | Sampling day 14 |
| S1 | Female, 29 | S1_d00 | S1_d07 | Absent |
| S2 | Female, 24 | S2_d00 | S2_d07 | S2_d14 |
| S3 | Female, 24 | S3_d00 | Absent | S3_d14 |
| S4 | Female, 24 | S4_d00 | S4_d07 | S4_d14 |
| S5 | Female, 26 | S5_d00 | S5_d07 | S5_d14 |
| S6 | Male, 26 | S6_d00 | S6_d07 | S6_d14 |
| S7 | Male, 26 | S7_d00 | S7_d07 | S7_d14 |
| S8 | Male, 25 | S8_d00 | S8_d07 | S8_d14 |

## Negative control swab (NCS)

One unused sterile swab was exposed to laboratory air for 5 min without contacting any surface and was processed as a negative control swab (NCS). The NCS underwent the same DNA extraction, library-preparation, sequencing, and bioinformatic workflow as the SDS samples. No separate extraction blank or library-preparation blank was included. In an exploratory sensitivity analysis, Decontam R package version 1.22.8, threshold = 0.1 was used, and contaminant=TRUE if $p < 0.1.

## DNA isolation

Total DNA was isolated using ZymoBIOMICS DNA Miniprep Kit (D4304; Zymo Research). Briefly, bacteria present in lysis solution were homogenized by bead beating for 20 seconds at 4500 RPM using Precellys Evolution homogenizer (Bertin Instruments). Homogenization was repeated 10 times with a pause of 20 seconds in-between each cycle. Following homogenization, supernatant was processed through a series of filters and washing steps according to the manufacturer's protocol. DNA was eluted in 50 µl DNase/RNase-free water provided with the kit and stored at -70°C until further processing. DNA concentration was assessed using a Qubit fluorometer with the Qubit 1× dsDNA High Sensitivity Assay Kit (cat. no. Q32854; Invitrogen) and a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific).

## Microbial community DNA (MCD) standard

We used 50 ng, 25 ng and 10 ng, in duplicate, of ZymoBIOMICS Microbial community DNA standard (D6305; Zymo Research) to assess our shotgun metagenomic sequencing and bioinformatic workflow. The MCD standard contained DNA from a defined mixture of Gram-positive and Gram-negative bacterial species and two fungal species. The MCD standard was introduced directly at the library-preparation step and therefore served to assess the sequencing and bioinformatic workflow, rather than the complete sampling and DNA-extraction workflow. The manufacturer-provided composition of the DNA standard is listed in Table S1.

## Library preparation and sequencing

Libraries were prepared using the Nextera XT DNA Sample Preparation Kit (Illumina Inc., San Diego, CA, USA). The low-input library-preparation protocol was adapted from Rinke et al. and described briefly (Rinke et al., 2016). A 1:10 diluted Amplicon Tagment Mix (ATM) was used for tagmentation. Samples were then incubated in a thermal cycler at 55°C for 5 min. Subsequently, tagmented DNA was amplified by polymerase chain reaction (PCR). To generate sufficient library material from low-input samples, 15 PCR cycles were used during library amplification (Nearing et al., 2021; Witzke et al., 2020).

Amplified libraries were purified with 1.6x Ampure XP beads. The quality of the purified libraries was assessed using the high sensitivity DNA kit on the Agilent 2100 Bioanalyzer (Figure S1). Subsequently, libraries were quantified through qPCR using the KAPA Library Quantification Kits (Roche), according to manufacturer's instructions. All libraries were sequenced on an Illumina NextSeq 550 instrument using a 150-cycle High Output kit to generate paired-end (2 × 75 bp) reads, with a target sequencing depth of approximately 10 million read pairs per sample. Base calling and quality scoring were performed as a first step including quality check on the on-board computer of the NextSeq 550.

# Data analysis

Most host-derived reads were removed using HoStile version 2.0.2 according to established method (Constantinides et al., 2023). Residual reads classified as Homo sapiens were excluded before taxonomic abundance analysis. Metagenomic classification was done with Kraken2(version 2.3.4) and Bracken (version 2.9) using the standard database: https://benlangmead.github.io/aws-indexes/k2. Counts were corrected with the Bracken 2.9 estimate abundance algorithm (https://ccb.jhu.edu/software/bracken/) with precomputed 100-mers included in the kraken2_PlusPF database. Counts were collected at the species and genus level. Estimated counts from all samples were merged using the Bracken combined outputs algorithm. Sample counts were normalized using DESeq2. The log2 of the normalized counts + 1 was used. Pearson correlation coefficients were calculated using the 20 most abundant bacterial genera across all SDS samples. Correlation plots were drawn using the R Corrplot package (https://cran.r-project.org/web/packages/corrplot/). Boxes around the inter-sample diagonals were added with the corrRect function.

## Results

# DNA libraries from low input DNA

DNA concentrations in all SDS and NCS extracts were below the detection limit of the Qubit assay (Table 1). Despite this, library preparation generated measurable sequencing libraries from the SDS samples. Because total DNA concentrations were below the detection limit and a substantial proportion of sequencing reads was classified as human, the amount of microbial DNA in the original extracts was inferred to be low. Libraries were amplified for 15 PCR cycles to obtain sufficient material for sequencing. Library fragment-size distributions are shown in Figure S1.

# Host DNA content

SDS samples from all eight individuals contained a variable proportion of reads classified as human, ranging from less than 20% to more than 80% of Kraken2-classified reads (Fig. 2). The proportion of reads classified as human varied both within and between individuals. Samples from S1, S3, and S4 generally had higher proportions of human reads, whereas samples from S6 and S8 had lower proportions (Fig. 2). Participant S2 showed the greatest within-individual variation in the proportion of reads classified as human. In an exploratory comparison involving three participants, dry-swab samples showed a lower proportion of reads classified as human than saline-moistened swab samples (Table S2). Because the comparison included only three participants and was not paired across multiple time points, this finding should be interpreted cautiously.

# Taxonomic profile of skin dry swab (SDS) samples

In addition to the quality-control analyses described above, we assessed similarity within and between individuals by calculating correlations based on the 20 most abundant bacterial genera across all SDS samples. Taxonomic classification was performed using Kraken2 and Bracken 2.9. Correlation analysis based on relative abundance estimates was influenced by variation in the proportion of reads classified as human, particularly for samples from S1, S3, and S4 (Fig. 3A). After DESeq2 normalization of Bracken-estimated bacterial counts, samples from the same individual appeared more similar to each other than to samples from other individuals (Fig. 3B). Given the limited number of participants and time points, this observation should be interpreted as exploratory.

Taxonomic profiling of SDS samples revealed that the most predominant bacterial genera were *Cutibacterium*, *Corynebacterium*, and *Staphylococcus.* (Fig. 4B and 5B, Table S2), whereas the most predominant bacterial species were *Cutibacterium acnes*, *Staphylococcus epidermidis* and *Corynebacterium ureicelerivorans.* (Fig. 4A and 5A, Table S2). Over the 14-day sampling period, samples from most participants were dominated by one bacterial genus, most commonly *Cutibacterium, Staphylococcus* or *Corynebacterium* (Figs. 4 and 5, Table S2). The NCS contained reads classified as *Homo sapiens* and low-level bacterial reads, with *Staphylococcus* as the most abundant bacterial genus (Fig. 4 and Table S2).

# Taxonomic profiling of microbial community DNA (MCD) standard

To assess the performance of library preparation, sequencing, and taxonomic classification, we processed a microbial community DNA standard (MCD) through the downstream workflow. MCD consisted of purified DNA from 10 distinct species, including eight bacterial and two yeast species. We performed the experiment in duplicates using three different amounts of total DNA: 50, 25 and 10 ng. This allowed us to test the most abundant species as 6, 3 and 1.2 ng, and the least abundant species as 1, 0.5 and 0.2 ng. The composition of MCD is listed in Table 2 and Table S1 (Sheet 1). The sequencing and classification workflow detected all species included in the MCD standard, including the lowest input amount tested. However, the observed taxonomic composition differed from the manufacturer's theoretical composition (Table 2). For example, in addition to *Bacillus subtilis* included in the MCD standard, the analysis assigned reads to three additional *Bacillus species*. Percent composition of all identified genera in the MCD is listed in Table S1 (Sheets 2 and 3).

Table 2
Expected and observed species-level composition of the microbial community DNA standard at three DNA input amounts.

| Species | Manufacturer's information | Sequencing outcome (% composition) | | | | | |
|---|---|---|---|---|---|---|---|
| | Composition (%) | Tx. Id | Total DNA (ng)/DNA standard technical replicate I or II | | | | |
| 50 – 1 | | | 50-II | 25-I | 25-II | 10-I | 10-II |
| *Pseudomonas aeruginosa* | 12 | 287 | 11 | 22.52 | 18.83 | 16.66 | 20.8 | 20.5 |
| *Escherichia coli* | 12 | 562 | 16.43 | 15.52 | 15.65 | 15.25 | 15.43 | 15.44 |
| *Salmonella enterica* | 12 | 28901 | 19.23 | 19.68 | 19.11 | 18.87 | 19.3 | 19.39 |
| *Limosilactobacillus fermentum* | 12 | 1613 | 8.15 | 8.53 | 8.24 | 8.24 | 8.64 | 8.65 |
| *Staphylococcus aureus* | 12 | 1280 | 9.39 | 6.05 | 7.57 | 8.28 | 6.78 | 6.58 |
| *Listeria monocytogenes* | 12 | 1639 | 7.71 | 5.26 | 6.22 | 6.89 | 5.79 | 5.89 |
| *Bacillus subtilis* | 12 | 1423 | 5.13 | 4.05 | 4.13 | 4.39 | 4.23 | 4.08 |
| *Enterococcus faecalis* | 12 | 1351 | 7.23 | 4.94 | 5.82 | 6.41 | 5.38 | 5.55 |
| *Saccharomyces cerevisiae* | 2 | 4932 | 1.62 | 1.15 | 1.34 | 1.47 | 1.24 | 1.26 |
| *Cryptococcus neoformans* | 2 | 5207 | 2.28 | 2.11 | 2.12 | 2.2 | 2.16 | 2.2 |
| *Bacillus spizizenii* | 0 | 96241 | 1.39 | 1.34 | 1.48 | 1.54 | 1.29 | 1.46 |
| *Bacillus intestinalis* | 0 | 1963032 | 3.12 | 2.04 | 2.17 | 2.81 | 2.3 | 2.24 |
| *Bacillus sp. KBS0812* | 0 | 2582827 | 1.13 | 0.94 | 1.34 | 0.8 | 0.84 | 1.06 |

## Discussion

This pilot study describes a feasible workflow for shotgun metagenomic profiling of DNA obtained from axillary skin dry swabs (SDS) containing very low amounts of total DNA. Sampling from a predefined 3 × 3 cm area in the centre of the left axilla was intended to improve consistency between longitudinal samples. Dry skin swabs may yield low amounts of microbial DNA and a high proportion of host DNA, which complicates downstream taxonomic profiling. The commonly used 16S rRNA gene sequencing approach provides limited species- and strain-level resolution and does not directly characterize the broader microbial gene content or functional potential of the sampled community (Durazzi et al., 2021; Yen and Johnson, 2021). Although 16S rRNA gene sequencing is useful for community profiling, the selected amplicon region may not provide reliable species- or strain-level resolution and does not directly characterize the broader microbial gene content or functional potential of the community (Matchado et al., 2024). Shotgun metagenomic sequencing has shown that the proportion of host DNA can exceed 90% of the total extracted DNA (Bjerre et al., 2019). Together, these constraints make direct shotgun metagenomic profiling of skin swabs challenging, particularly when host DNA depletion is not performed. Therefore, we developed and evaluated a modified shotgun metagenomic workflow comprising axillary dry-swab sampling, DNA extraction, low-input library preparation, sequencing, and bioinformatic taxonomic profiling. We adapted a previously published low-input shotgun library-preparation approach, originally developed for microscale marine metagenomics, to DNA obtained from SDS samples (Rinke et al., 2016). DNA isolation resulted in exceptionally low amounts of DNA, and sequencing analysis showed that it consisted of considerable amounts of human DNA. Because DNA concentrations were below the Qubit detection limit, extracted DNA was introduced into library preparation without normalization to a measured DNA input concentration. By modifying the library-preparation protocol to use 15 PCR cycles, it was possible to generate

measurable sequencing libraries from exceptionally low amounts of DNA. It has been shown that DNA extraction and host depletion methods significantly impact and potentially bias bacterial detection (Ganda et al., n.d.). Host DNA depletion is not always recommended for skin microbiome research due to some loss of bacteria and decrease in the relative abundances of Gram-negative microbes (Marquet et al., 2022). Metagenomic sequencing in combination with some host DNA depletion methods of skin samples may be difficult (Ahannach et al., 2021).

In our study, the swab following sampling was inserted in a tube containing DNA/RNA shield (Zymo Research). Because DNA/RNA shield lyses both host and microbial cells upon contact to preserve DNA, the samples lack the intact bacterial cells required for selective host cells lysis procedure (Cole et al., 2025). Consequently, a host DNA depletion step could not be performed.

We isolated DNA from armpit-swabs from eight healthy individuals on days 0, 7 and 14. It has been shown that longitudinal sampling is especially important for studying inter- and intra-individual stability or difference in microbiota over a period (Olsson et al., 2022). Our analysis showed higher genus-level correlation within individuals than between individuals over two weeks. This pattern is consistent with relative within-individual stability; however, it does not by itself validate the workflow because the number of participants, time points, and negative controls was limited. Consistent with previous reports of the axillary microbiome, the most abundant bacterial taxa belonged primarily to the phyla Actinobacteria and Firmicutes (Serghiou et al., 2023; Troccaz et al., 2015; Urban et al., 2016). Our analysis suggests relative short-term stability of inferred axillary bacterial profiles over this short duration. Within individual variations over the study period may be attributed either to natural biological variations or could simply be a common artifact of WGS when working with extremely low input DNA samples (Serghiou et al., 2023). No sex-specific analyses were performed because the study was not designed or powered to assess sex-associated differences in axillary microbiota (Troccaz et al., 2015).

Low-abundance reads assigned to the genus *Salmonella* were detected in several samples, including the negative-control swab. Because *Salmonella* is not generally regarded as a typical resident of healthy axillary skin and was also detected in the negative control, these reads are likely to represent background contamination or taxonomic misclassification rather than true skin colonization. Contamination is a major concern in sequencing studies of low biomass samples (Fierer et al., 2025; Salter et al., 2014).

Our microbial community DNA standard (MCD) sequencing results identify all microbial species within the samples even at the lowest concentration. However, the MCD composition of our sample varied from the manufacturer's composition. It was in part due to the inability to distinguish very closely related species, e.g. *Bacillus* species. While 16S sequencing analysis usually misattribute highly similar sequences to the same species (Devanga Ragupathi et al., 2018), the analysis assigned some reads to additional closely related *Bacillus* species, suggesting that species-level assignments should be interpreted cautiously

The air-exposed negative-control swab contained low levels of reads assigned to several bacterial genera, including *Staphylococcus*. Because no extraction blank or library-preparation blank was included, these findings cannot distinguish laboratory-air contamination from reagent- or workflow-associated contamination. A low-level of contamination is common in skin samples which yield very low levels of input bacteria (Serghiou et al., 2023). Because *Staphylococcus* was detected in the NCS, the relative abundance of this genus in SDS samples may have been influenced by background contamination. However, the magnitude of this effect could not be quantified because only one negative control was included. In an exploratory sensitivity analysis using Decontam in our study, *Staphylococcus aureus* was identified as a potential contaminant and excluded. Its exclusion did not change the identity of the three most abundant taxa across samples. The MCD and SDS samples should therefore be interpreted as distinct controls addressing different potential sources of bias.

## Conclusions

This pilot study indicates that dry swab sampling can provide exploratory shotgun metagenomic profiles of the axillary skin microbiome, despite the low biomass and DNA concentrations being below the detection limit of fluorometric quantification.

Microbiome profiles were relatively stable within individuals across three sampling time points over 14 days, supporting the potential utility of dry swabs for longitudinal sampling of the axilla.

Our findings provide proof-of-concept that dry swabbing may be a practical alternative to wet swabbing for future shotgun metagenomic studies of the skin microbiome. However, only a small exploratory comparison with wet swabs was performed in three participants, and the workflow has not been evaluated for other skin sites. Further validation in larger studies, including several negative controls and sampling from additional skin surfaces, is needed to assess the broader applicability of the method.

## Declarations

*Ethics approval and consent to participate*

The study was conducted according to the guidelines of the Declaration of Helsinki. Approvals were granted by the Regional Committee of Medical and Health Research Ethics of Northern Norway (ref no: 50072/2019). Written informed consent was obtained from all participants.

*Consent for publication*

Not applicable

*Availability of data and materials*

The data generated or analyzed during this study are included in this manuscript and its supplementary information files. Sequencing data will be available through NCBI BioProject PRJNA1365519 following SRA approval. https://www.ncbi.nlm.nih.gov/bioproject/1365519

*Competing interests*

The authors declare that they have no competing interests.

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Conceptualization: BS, CGF, RHP, AMH, ØG. Data Curation: BS, CGF, RHP. Formal Analysis: BS, CGF. Funding acquisition: ØG. Investigation: BS, CGF, RHP. Methodology: BS, CGF, RHP. Project administration: AMH. Resources: RHP, ØG. Software: CGF. Supervision: AMH, ØG. Visualization: BS, CGF. Writing- original draft: BS. Writing-review and editing: all authors.

*Declaration of generative AI and AI-assisted technologies in the manuscript preparation process*

During preparation of this manuscript, the authors used ChatGPT (OpenAI) to assist with language editing, improvement of readability, identification of internal inconsistencies, and refinement of the presentation of the manuscript. The AI tool was not used to generate data, perform data analysis, interpret results, generate references, or draw scientific conclusions. All AI-assisted text was critically reviewed, verified, and revised by the authors, who take full responsibility for the accuracy, originality, and integrity of the manuscript.

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## References


1. Ahannach S, Delanghe L, Spacova I, Wittouck S, Van Beeck W, De Boeck I, Lebeer S. Microbial enrichment and storage for metagenomics of vaginal, skin, and saliva samples. iScience. 2021;24:103306. https://doi.org/10.1016/j.isci.2021.103306.
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;43:906–11. https://doi.org/10.1111/1346-8138.13245.
3. Alekseyenko AV, Perez-Perez GI, De Souza A, Strober B, Gao Z, Bihan M, Li K, Methé BA, Blaser MJ. Community differentiation of the cutaneous microbiota in psoriasis. Microbiome. 2013;1:31. https://doi.org/10.1186/2049-2618-1-31.
4. Bay L, Barnes CJ, Fritz BG, Thorsen J, Restrup MEM, Rasmussen L, Sørensen JK, Hesselvig AB, Odgaard A, Hansen AJ, Bjarnsholt T. Univers Dermal Microbiome Hum Skin mBio. 2020;11:e02945–19. https://doi.org/10.1128/mBio.02945-19.
5. Bjerre RD, Hugerth LW, Boulund F, Seifert M, Johansen JD, Engstrand L. Effects of sampling strategy and DNA extraction on human skin microbiome investigations. Sci Rep. 2019;9:17287. https://doi.org/10.1038/s41598-019-53599-z.
6. Brumfield KD, Huq A, Colwell RR, Olds JL, Leddy MB. Microbial resolution of whole genome shotgun and 16S amplicon metagenomic sequencing using publicly available NEON data. PLoS ONE. 2020;15. https://doi.org/10.1371/JOURNAL.PONE.0228899.
7. Byrd AL, Belkaid Y, Segre JA. The human skin microbiome. Nat Rev Microbiol. 2018;16:143–55. org/10.1038/nrmicro.2017.157. https://doi.org/http://dx.doi.
8. Cole AL, Marzahn MR, Valdespino EL, Patel R, Cole AM. Nasal microbiome inhabitants with anti-Staphylococcus aureus activity. Microbiol Spectr. 2025;13:e01024–25. https://doi.org/10.1128/spectrum.01024-25.
9. Constantinides B, Hunt M, Crook DW. Hostile: accurate decontamination of microbial host sequences. Bioinformatics. 2023;39:btad728. https://doi.org/10.1093/bioinformatics/btad728.
10. Devanga Ragupathi NK, Sethuvel M, Inbanathan DP, Veeraraghavan FY, B. Accurate differentiation of Escherichia coli and Shigella serogroups: challenges and strategies. New Microbes New Infections. 2018;21:58–62. https://doi.org/10.1016/j.nmni.2017.09.003.
11. Durazzi F, Sala C, Castellani G, Manfreda G, Remondini D, De Cesare A. Comparison between 16S rRNA and shotgun sequencing data for the taxonomic characterization of the gut microbiota. Sci Rep. 2021;11:3030. https://doi.org/10.1038/s41598-021-82726-y.
12. Fierer N, Leung PM, Lappan R, Eisenhofer R, Ricci F, Holland SI, Dragone N, Blackall LL, Dong X, Dorador C, Ferrari BC, Goordial J, Holmes SP, Inagaki F, Korem T, Li SS, Makhalanyane TP, Metcalf JL, Nagarajan N, Orsi WD, Shanahan ER, Walker AW, Weyrich LS, Gilbert JA, Willis AD, Callahan BJ, Shade A, Parkhill J, Banfield JF, Greening C. Guidelines for preventing and reporting contamination in low-biomass microbiome studies. Nat Microbiol. 2025;10:1570–80. https://doi.org/10.1038/s41564-025-02035-2.
13. Ganda E, Beck KL, Haiminen N, Silverman JD, Kawas B, Cronk BD, Anderson RR, Goodman LB, Wiedmann M. n.d. DNA Extraction and Host Depletion Methods Significantly Impact and Potentially Bias Bacterial Detection in a Biological Fluid. mSystems 6, e00619-21. https://doi.org/10.1128/mSystems.00619-21
14. Grice EA, Kong HH, Renaud G, Young AC, Bouffard GG, Blakesley RW, Wolfsberg TG, Turner ML, Segre JA. A diversity profile of the human skin microbiota. Genome Res. 2008;18:1043–50. https://doi.org/10.1101/GR.075549.107.
15. Jo JH, Harkins CP, Schwardt NH, Portillo JA, Zimmerman MD, Carter CL, Hossen MA, Peer CJ, Polley EC, Dartois V, Figg WD, Moutsopoulos NM, Segre JA, Kong HH. Alterations of human skin microbiome and expansion of antimicrobial resistance after systemic antibiotics. Sci Transl Med. 2021;13. https://doi.org/10.1126/SCITRANSLMED.ABD8077/SUPPL_FILE/SCITRANSLMED.ABD8077_DATA_FILES_S1_TO_S5.ZIP.

16. Johnson JS, Spakowicz DJ, Hong BY, Petersen LM, Demkowicz P, Chen L, Leopold SR, Hanson BM, Agresta HO, Gerstein M, Sodergren E, Weinstock GM. 2019. Evaluation of 16S rRNA gene sequencing for species and strain-level microbiome analysis 10, 1–11.
17. Kong HH, Andersson B, Clavel T, Common JE, Jackson SA, Olson ND, Segre JA, Traidl-Hoffmann C. Performing Skin Microbiome Research: A Method to the Madness. J Invest Dermatology. 2017;137:561–8. https://doi.org/10.1016/J.JID.2016.10.033.
18. Kong HH, Segre JA. Skin Microbiome: Looking Back to Move Forward. J Invest Dermatology. 2012;132:933–9. https://doi.org/10.1038/JID.2011.417.
19. Marquet M, Zöllkau J, Pastuschek J, Viehweger A, Schleußner E, Makarewicz O, Pletz MW, Ehricht R, Brandt C. Evaluation of microbiome enrichment and host DNA depletion in human vaginal samples using Oxford Nanopore's adaptive sequencing. Sci Rep. 2022;12:4000. https://doi.org/10.1038/s41598-022-08003-8.
20. Matchado MS, Rühlemann M, Reitmeier S, Kacprowski T, Frost F, Haller D, Baumbach J, List M. On the limits of 16S rRNA gene-based metagenome prediction and functional profiling. Microb Genom. 2024;10:001203. https://doi.org/10.1099/mgen.0.001203.
21. Melendez JH, Frankel YM, An AT, Williams L, Price LB, Wang NY, Lazarus GS, Zenilman JM. Real-time PCR assays compared to culture-based approaches for identification of aerobic bacteria in chronic wounds. Clin Microbiol Infect. 2010;16:1762–9. https://doi.org/10.1111/J.1469-0691.2010.03158.X.
22. Nearing JT, Comeau AM, Langille MGI. Identifying biases and their potential solutions in human microbiome studies. Microbiome. 2021;9:113. https://doi.org/10.1186/s40168-021-01059-0.
23. Nowicka D, Chilicka K, Dzieńdziora-Urbińska I. Host-Microbe Interaction on the Skin and Its Role in the Pathogenesis and Treatment of Atopic Dermatitis. Pathogens. 2022;11:71. https://doi.org/10.3390/pathogens11010071.
24. Ogai K, Nagase S, Mukai K, Iuchi T, Mori Y, Matsue M, Sugitani K, Sugama J, Okamoto S. A Comparison of Techniques for Collecting Skin Microbiome Samples: Swabbing Versus Tape-Stripping. Front Microbiol. 2018;9:2362. https://doi.org/10.3389/fmicb.2018.02362.
25. Oh J, Byrd AL, Deming C, Conlan S, Kong HH, Segre JA, Barnabas B, Blakesley R, Bouffard G, Brooks S, Coleman H, Dekhtyar M, Gregory M, Guan X, Gupta J, Han J, Ho SL, Legaspi R, Maduro Q, Masiello C, Maskeri B, McDowell J, Montemayor C, Mullikin J, Park M, Riebow N, Schandler K, Schmidt B, Sison C, Stantripop M, Thomas J, Thomas P, Vemulapalli M, Young A. Biogeography and individuality shape function in the human skin metagenome. Nature. 2014;514:59–64. https://doi.org/10.1038/NATURE13786.
26. Olsson LM, Boulund F, Nilsson S, Khan MT, Gummesson A, Fagerberg L, Engstrand L, Perkins R, Uhlén M, Bergström G, Tremaroli V, Bäckhed F. Dynamics of the normal gut microbiota: A longitudinal one-year population study in Sweden. Cell Host Microbe. 2022;30:726–e7393. https://doi.org/10.1016/j.chom.2022.03.002.
27. Ranjan R, Rani A, Metwally A, McGee HS, Perkins DL. Analysis of the microbiome: Advantages of whole genome shotgun versus 16S amplicon sequencing. Biochem Biophys Res Commun. 2016;469:967–77. https://doi.org/10.1016/J.BBRC.2015.12.083.
28. Rhoads DD, Cox SB, Rees EJ, Sun Y, Wolcott RD. Clinical identification of bacteria in human chronic wound infections: Culturing vs. 16S ribosomal DNA sequencing. BMC Infect Dis. 2012a;12:1–8. https://doi.org/10.1186/1471-2334-12-321/FIGURES/4.
29. Rhoads DD, Wolcott RD, Sun Y, Dowd SE. 2012b. Comparison of Culture and Molecular Identification of Bacteria in Chronic Wounds. International Journal of Molecular Sciences 2012, Vol. 13, Pages 2535–2550 13, 2535–2550. https://doi.org/10.3390/IJMS13032535
30. Richardson M, Gottel N, Gilbert JA, Lax S. 2019. Microbial Similarity between Students in a Common Dormitory Environment Reveals the Forensic Potential of Individual Microbial Signatures. mBio 10. 10.1128/mbio.01054-19. https://doi.org/10.1128/mbio.01054-19.

31. Rinke C, Low S, Woodcroft BJ, Raina JB, Skarshewski A, Le XH, Butler MK, Stocker R, Seymour J, Tyson GW, Hugenholtz P. Validation of picogram- and femtogram-input DNA libraries for microscale metagenomics. PeerJ. 2016;4:e2486. https://doi.org/10.7717/peerj.2486.
32. Ruuskanen MO, Vats D, Potbhare R, RaviKumar A, Munukka E, Ashma R, Lahti L. Towards standardized and reproducible research in skin microbiomes. Environ Microbiol. 2022;24:3840–60. https://doi.org/10.1111/1462-2920.15945.
33. Salter SJ, Cox MJ, Turek EM, Calus ST, Cookson WO, Moffatt MF, Turner P, Parkhill J, Loman NJ, Walker AW. Reagent and laboratory contamination can critically impact sequence-based microbiome analyses. BMC Biol. 2014;12:87. https://doi.org/10.1186/s12915-014-0087-z.
34. Schommer NN, Gallo RL. Structure and function of the human skin microbiome. Trends Microbiol. 2013;21:660–8. https://doi.org/10.1016/j.tim.2013.10.001.
35. Serghiou IR, Baker D, Evans R, Dalby MJ, Kiu R, Trampari E, Phillips S, Watt R, Atkinson T, Murphy B, Hall LJ, Webber MA. An efficient method for high molecular weight bacterial DNA extraction suitable for shotgun metagenomics from skin swabs. Microb Genomics. 2023;9. https://doi.org/10.1099/mgen.0.001058.
36. Timm CM, Loomis K, Stone W, Mehoke T, Brensinger B, Pellicore M, Staniczenko PPA, Charles C, Nayak S, Karig DK. Isolation and characterization of diverse microbial representatives from the human skin microbiome. Microbiome. 2020;8:1–12. https://doi.org/10.1186/S40168-020-00831-Y/TABLES/1.
37. Troccaz M, Gaïa N, Beccucci S, Schrenzel J, Cayeux I, Starkenmann C, Lazarevic V. Mapping axillary microbiota responsible for body odours using a culture-independent approach. Microbiome. 2015;3:3. https://doi.org/10.1186/s40168-014-0064-3.
38. Urban J, Fergus DJ, Savage AM, Ehlers M, Menninger HL, Dunn RR, Horvath JE. The effect of habitual and experimental antiperspirant and deodorant product use on the armpit microbiome. PeerJ. 2016;4:e1605. https://doi.org/10.7717/peerj.1605.
39. Weyrich LS, Dixit S, Farrer AG, Cooper AJ, Cooper AJ. The skin microbiome: Associations between altered microbial communities and disease. Australas J Dermatol. 2015;56:268–74. https://doi.org/10.1111/ajd.12253.
40. Witzke MC, Gullic A, Yang P, Bivens NJ, Adkins PRF, Ericsson AC. Influence of PCR cycle number on 16S rRNA gene amplicon sequencing of low biomass samples. J Microbiol Methods. 2020;176:106033. https://doi.org/10.1016/j.mimet.2020.106033.
41. Yen S, Johnson JS. Metagenomics: a path to understanding the gut microbiome. Mamm Genome. 2021;32:282–96. https://doi.org/10.1007/s00335-021-09889-x.
42. Zeeuwen PLJM, Boekhorst J, van den Bogaard EH, de Koning HD, van de Kerkhof PMC, Saulnier DM, van Swam II, van Hijum SAFT, Kleerebezem M, Schalkwijk J, Timmerman HM. Microbiome dynamics of human epidermis following skin barrier disruption. Genome Biol. 2012;13. https://doi.org/10.1186/GB-2012-13-11-R101.

## Figures

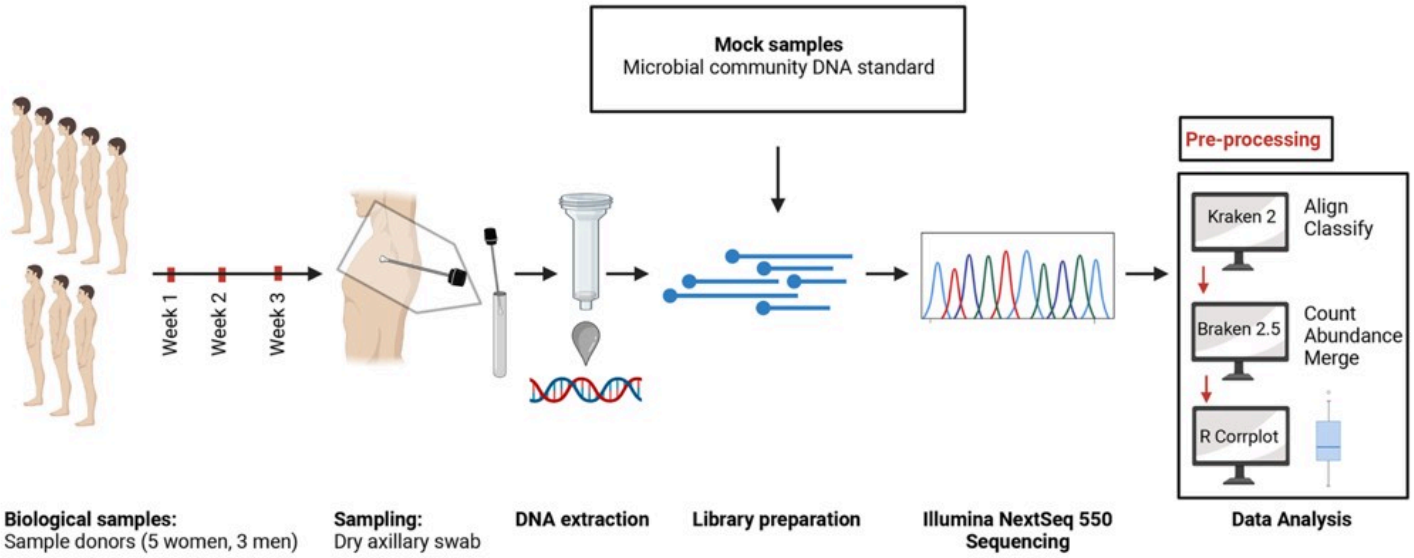


**Figure 1**

**Experimental design for the collection of bacterial samples from axillary skin by dry swabbing for metagenomic analysis.** Isolated DNA from skin dry swabs (SDS) was directly used for library preparation and sequencing because DNA concentrations were below the fluorometric detection limit and could not be used for input normalization. In parallel, a microbial community DNA (MCD) standard was added to the sequencing workflow and processed directly for library preparation. Downstream analysis and establishment of metagenomic profiles were conducted with Kraken 2 and Bracken 2. The figure was created using BioRender.com.

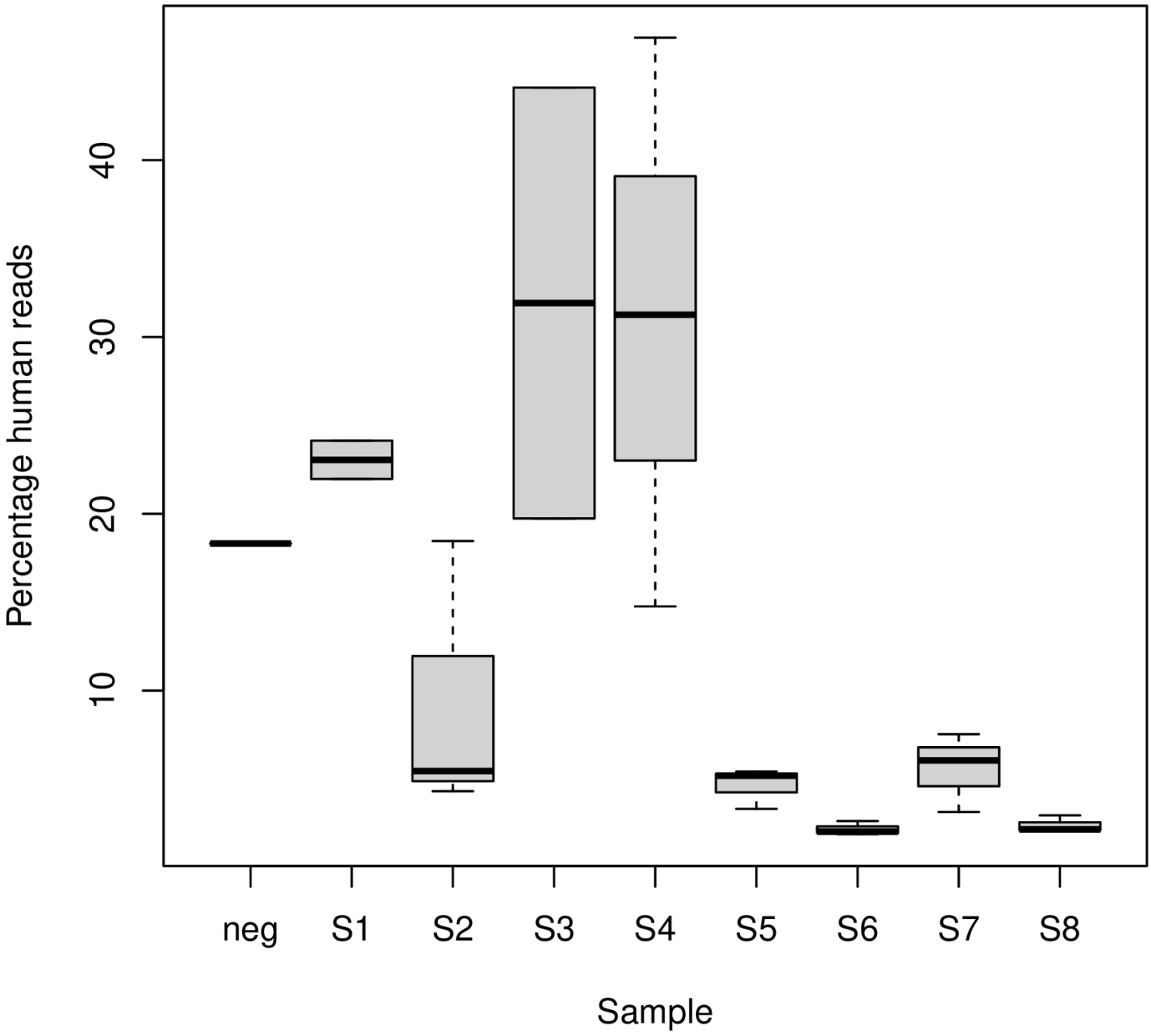


**Figure 2**

**Proportion of reads classified as *Homo sapiens* in each SDS sample.** Boxplot showing the fraction of Kraken2 classified and Bracken corrected reads that were classified as *Homo sapiens* for each individual sample (S1-S8). The y-axis indicates the percentage of reads classified as human.

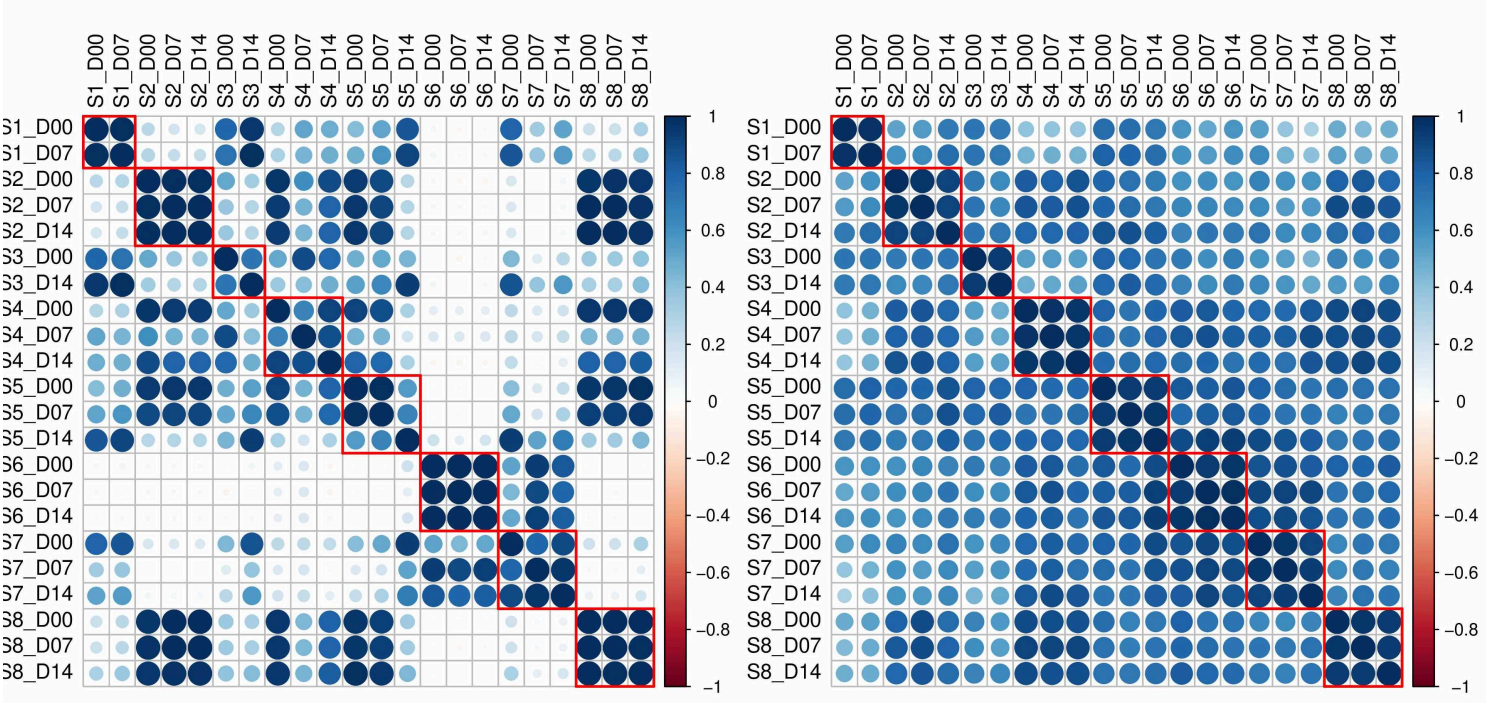


**Figure 3**

**Correlation of genus-level taxonomic profiles across longitudinal SDS samples.** (A) Correlation heatmap based on relative abundances of the 20 most abundant bacterial genera estimated by Kraken2/Bracken. (B) Pearson correlation heatmap based on DESeq2-normalized genus-level counts. Red boxes indicate samples from the same participant collected on days 0, 7, and 14. Sample labels indicate participant and time point; for example, S1_d00 denotes participant 1 at day 0.

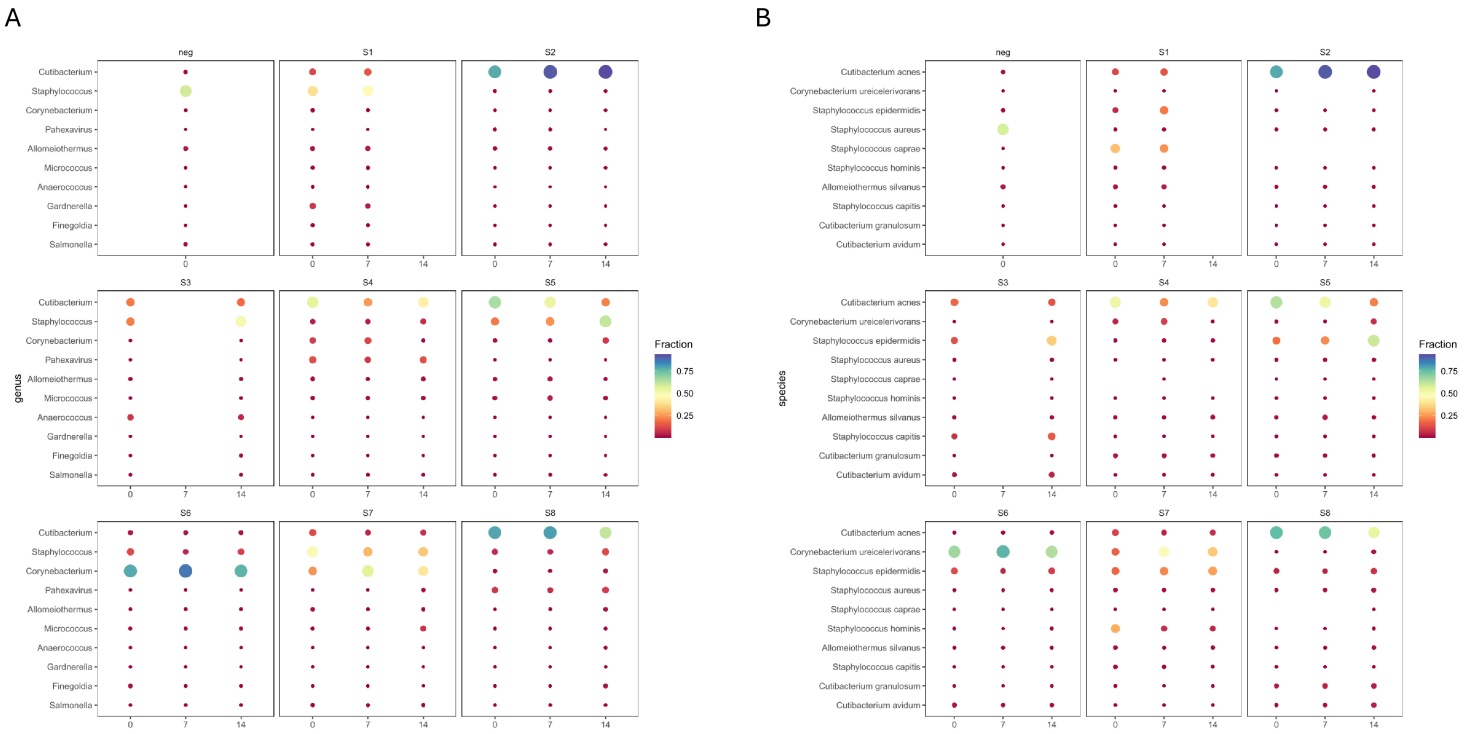


**Figure 4**

**Taxonomic profiling of skin dry swab (SDS) samples using a bubble plot.** The figure shows the relative abundance of the ten most abundant bacterial genera (A) and species (B) in samples from individuals S1–S8 and the negative control (neg). The x-axis shows the sampling days (0, 7 and 14), and the panels show the individual participants. Bubble size and colour represent the fraction of bacterial reads assigned to each species after removal of human reads.

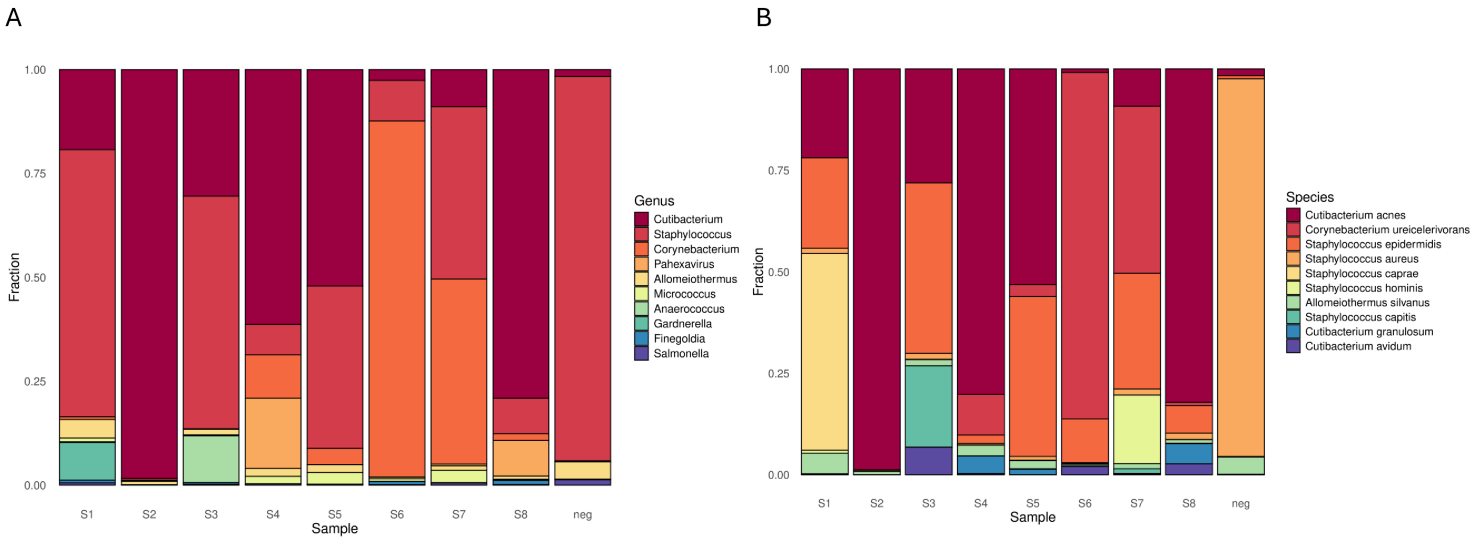


**Figure 5**

T**axonomic profiling of skin dry swab (SDS) samples using stacked bar charts.** The x-axis shows the samples from individuals S1–S8 and the negative control (neg), and the y-axis shows the fraction of classified reads after removal of human reads. The bars represent the relative abundance of the ten most abundant bacterial genera (A) and species (B). For individuals S1–S8, values represent the mean across sampling days 0, 7 and 14. Different genera and species are indicated by different colours, as shown in the legend.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- TableS1.xlsx
- TableS2.xlsx
- SupplementaryMaterialLegends.docx