Title: Comparing Skin Microbiome Compositions Across Various Body Locations and Regions
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INTRODUCTION

The human skin is colonized by a diverse community of microorganisms collectively known as the skin microbiome. Every individual's skin microbiome is unique, with microbial populations continuously shifting in response to various stimuli: particularly, geography and diet [1]. The protective role of the skin microbiome against pathogens is well-established [2]. Studies have found associations between imbalances in the skin microbiome and conditions such as acne, eczema, and psoriasis, as well as diabetes and autoimmune disorders [3]. Skin microbiome can also influence the efficacy of certain cosmetic products and can determine the skin's response to different topical treatments [4]. Given its broad role in maintaining human health, there is a clear need for deeper exploration of the skin microbiome through targeted research. Today, thanks to recent advances in gene sequencing technologies and computational tools for analyzing genomic data, we can explore the skin microbiome in various contexts. An important aspect of skin microbiome research is spatial microbial diversity. For public health researchers, it is crucial to better understand how microbial compositions differ across various parts of the human skin to find patterns in affected groups and lay the foundations for personalized treatments for skin disease. Many existing studies have tracked how one's skin microbiome changes as they age. Others have sought to quantify richness of specific disease-related genera at multiple body sites [5]. However, few studies have provided a *broad* snapshot of the complete skin microbiome in various locations: key information in identifying the specialized roles of certain bacteria in maintaining skin health across diverse environments. The first aim of our study is to **sample and compare the microbiome of five skin locations** in a population of George Washington University (GWU) graduate students to assess spatial microbial diversity. Our secondary aim is to use these data to **test the "grandma hypothesis,"** which theorizes that less-washed body regions will have higher microbial diversity than more-washed regions.

MATERIALS AND METHODS

To collect sample microbial data, 15 healthy graduate students at GWU self-swabbed five locations of their skin in January of 2023. All participants provided written email consent using the GWU IRB-approved 2022 Informed Consent Document prior to self-swabbing. Body locations included: navel (BE), back of both calves (CA), behind both ears (EA), inner forearms (FA), and between all toes (TO). All samples from the BE, EA, and TO locations were assigned to the "less-washed" region for testing the grandma hypothesis. All CA and FA samples were assigned to the "more-washed" region. Mimicking the sampling protocols of the NIH Human Microbiome Project, each location was swabbed twice by rubbing separate, sterilized, Catch-AllTM Sample Collection swabs across the area for 30 seconds, before immersing in SCF-1 buffer solution and storing at -80˚C. All DNA was extracted from the samples using the ZymoBIOMICS DNA Miniprep Kit and quality-checked using the NanoDrop 2000 UV-Vis Spectrophotometer. Microbial DNA from the V4 region of the 16S rRNA gene alone was amplified by PCR with controls and resulting amplicons were sequenced by gel electrophoresis using the Agilent 2100 Bioanalyzer. Amplicons were assembled into libraries via the sequencing-by-synthesis protocol using a single run of the Illumina MiSeq sequencing platform [6]. The DADA2 software was used to correct errors in Ilumina amplicon sequencing [7]. The data were sorted into four FASTQ files containing Amplicon Sequence Variant (ASV) data, metadata, a Newick tree, and SILVA taxonomy. In total, we obtained 1,481,298 sequences across 75 initial samples and 9,443 ASVs pre-filtering.

In MicrobiomeAnalyst, we excluded all five samples with less than 1,000 reads and removed singletons [8]. We also applied a 10% threshold for both "Mean Abundance Value" and "Inter-quantile Range." After filtering, 243 ASVs remained across 70 samples in the dataset. The data were not rarefied but were scaled by Total Sum Scaling. The "Interactive Pie Chart" tool was used to assess percentage microbial composition for "all samples (sum)," setting taxonomy level to phylum and merging "small taxa with counts < 10." In the "Core Microbiome" tool, taxonomic level was set first to phylum and then to genus, while sample prevalence and relative abundance were set to 80% and 0.01% respectively. In the "Alpha Diversity" tool, Shannon Index and Mann-Whitney/Kruskal-Wallis were selected as the Diversity Measure and Statistical Method parameters, respectively. In the "Beta Diversity" tool, PCoA, Weighted UniFrac Distance, and ANOSIM were selected as parameters for Ordination, Distance, and Statistical Methods respectively at the feature-level. Location, T-Test/ANOVA, and 0.05 were chosen as parameters for Experimental Factor, Statistical Method, and Adjusted

p-Value Cutoff respectively in the “Single-Factor Analysis” tool at the phylum-level. Multiple test correction was conducted by FDR.

RESULTS

In pursuit of our two overarching research aims, we used MicrobiomeAnalyst and the methods described above to assess skin microbiome diversity across all five locations and both regions. **Fig 1.** shows that bacteria from the Firmicutes phylum made up the majority of the microbial composition across all skin samples in the study. Actinobacteria and Proteobacteria were the second and third most prevalent phyla in the dataset, respectively. In **Fig 2A.,** we see evidence that only Firmicutes and Actinobacteria were ubiquitous across all samples, therefore representing the core phylum-level microbiome in this study. **Fig 2B.** shows that only Staphylococcus and Corynebacterium were ubiquitous, representing the core genus-level microbiome. Key to our assessment of spatial differences in skin microbial composition, bacteria from these two phyla and genera were present in all five body locations. They were also present in both less- and more-washed regions. **Fig 3A.**, shows higher median and minimum Shannon Indices in CA and FA compared to the three other locations, indicating greater richness and evenness (i.e. diversity) in the distribution of microbial species at these locations. **Fig 3B.**, corroborates these patterns, also showing higher median and minimum Shannon Index scores in more-washed compared to less-washed regions. All Shannon Index scores in both alpha diversity plots were significant at a 99.99% threshold ($p < 0.00005$). In **Fig 4A.**, FA and CA samples appear to be clustered closer together implying that ASVs in these two locations are more similar to each other than to other locations. BE samples were either most-closely clustered around TO or EA samples. This plot has $p < 0.001$, indicating that at least one location’s microbial composition is significantly different from the others. **Fig 4B.** shows how samples from more-washed areas are clustered far closer to each other than to samples from less-washed areas (and vice versa). Since $p < 0.005$, this indicates significant differences in microbial composition between the two regions. **Table 1A.** identifies Proteobacteria, Firmicutes, and Actinobacteria as the only microbial phyla that vary significantly (FDR-adjusted $p < 0.05$) in abundance across locations. **Table 1B.** identifies 12 genera–Streptococcus, Corynebacterium, Staphylococcus, Shigella, Lawsonella, Noviherbaspirillum, Anaerococcus, Paenibacillus, Micrococcus, Bacillus, Pseudomonas, and Gemella–as varying significantly in abundance across regions.

DISCUSSION

Schommer and Gallo found Firmicutes, Actinobacteria, and Proteobacteria–the same three phyla we reported–to be the most prevalent in human skin microbiome; however, they found Actinobacteria to be more prevalent than Firmicutes [9]. Conversely, Ellis et al. found that “Firmicutes usually predominate in the [skin] microbiome of adults” [10]. It seems that, the *order* of most prevalent phyla in the skin microbiome–but not the phyla themselves–varies based on specific study design. Our second set of results corroborates the findings from Grice and Segre that Firmicutes and Actinobacteria, as well as Staphylococcus and Corynebacterium, belong to the core phylum-level and genus-level skin microbiome respectively. Grice and Segre also found 1 other phylum and genus each as members of the core microbiome. Since their study included samples from several different countries, greater diversity in geography and diet may have led to their slightly broader core microbiome findings [11]. Our third set of results **shows evidence against the grandma hypothesis**, as more-washed locations and regions have *more* microbial diversity than less-washed counterparts. Hwang et al. also found more-washed areas of skin to have greater microbial diversity; unlike the association presented in the grandma hypothesis, less-washed regions actually allow buildup of certain phyla, leading to less-diverse and unhealthier skin microbiomes [4]. Our fourth set of results is also consistent with these findings in Hwang et al., with more-washed locations clustering closer together than less-washed locations in terms of beta diversity [4]. Our last set of results found significantly varying abundances of phyla and genera across locations and regions, **showing evidence for spatial diversity of skin microbial composition**. These results are consistent with several findings from Grice and Segre; for example, they also found Proteobacteria to be significantly more abundant in the “volar forearm” compared to the “umbilicus” and “toe web space” [11].

CONCLUSIONS

Our results provide strong evidence for significantly varying skin microbial compositions across body locations. We also found higher microbial diversity in more-washed regions compared to less-washed regions.

TABLES and FIGURES

Fig 1. Pie chart showing relative microbial composition of each phylum across all 70 samples in the filtered ASV dataset.

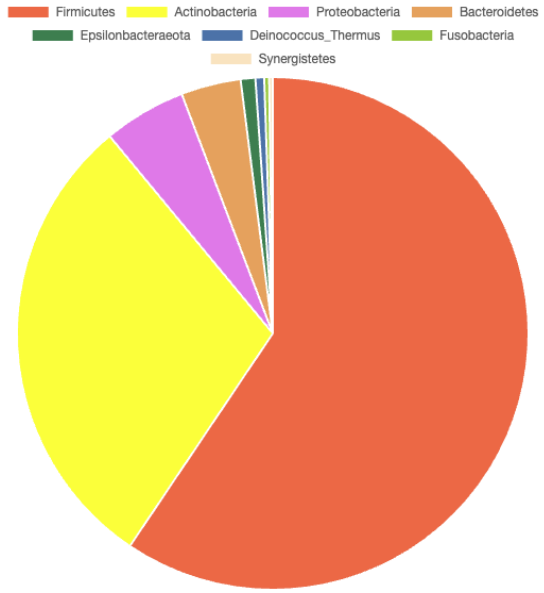


Fig 2. Members of the core microbiome with sample prevalence ≥ 80% and relative abundance ≥ 0.01%. (A) phylum-level core microbiome; (B) genus-level core microbiome.

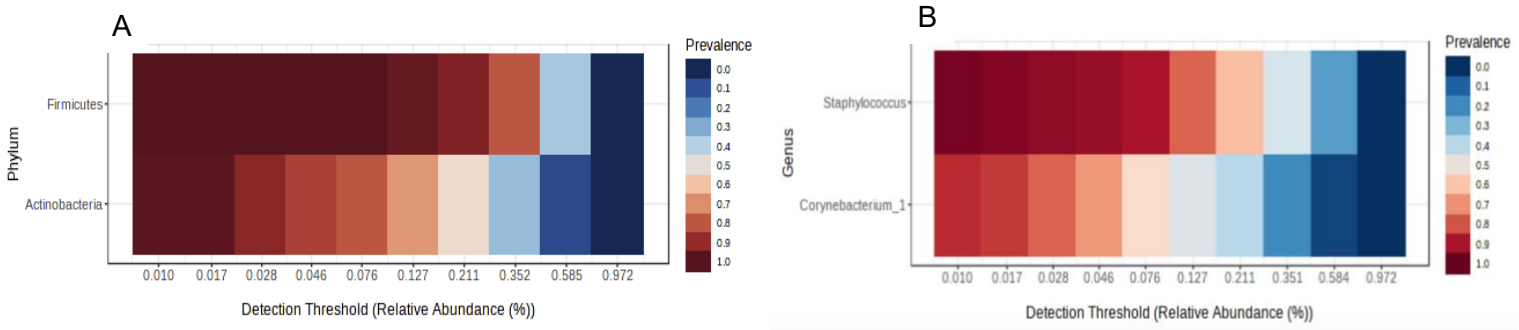


Fig 3. Visualizing alpha diversity of skin microbiome using the Shannon Index and Mann-Whitney/Kruskal-Wallis statistical method. (A) by location (BE = navel, CA = calves, EA = ears, FA = forearms, TO = toes); (B) by region (less = less-washed regions, more = more-washed regions).

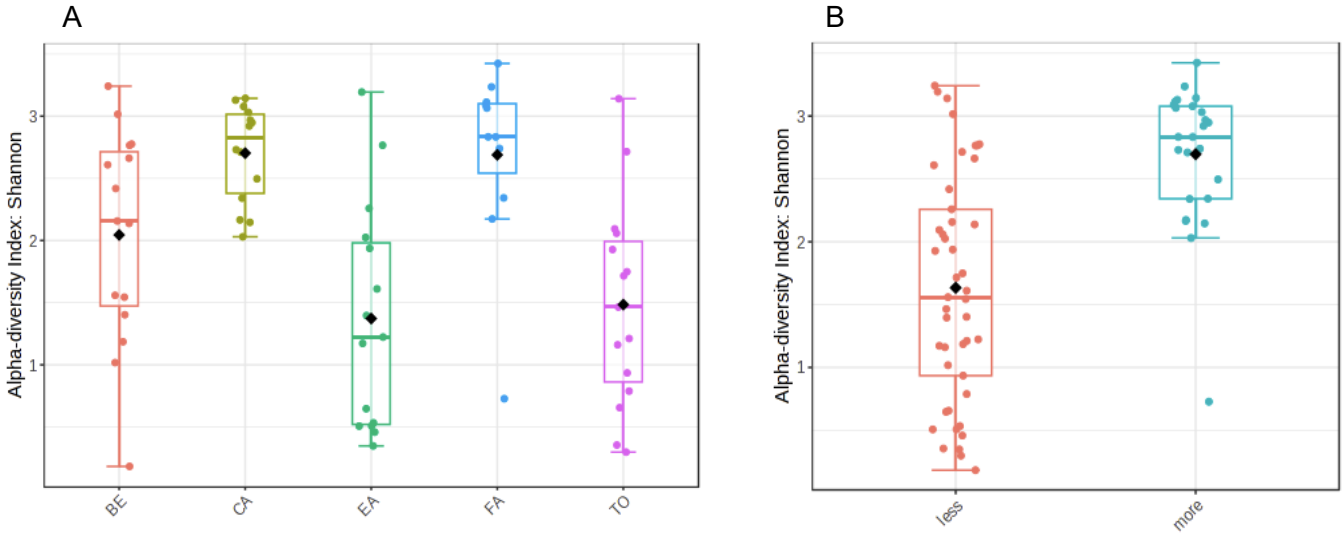
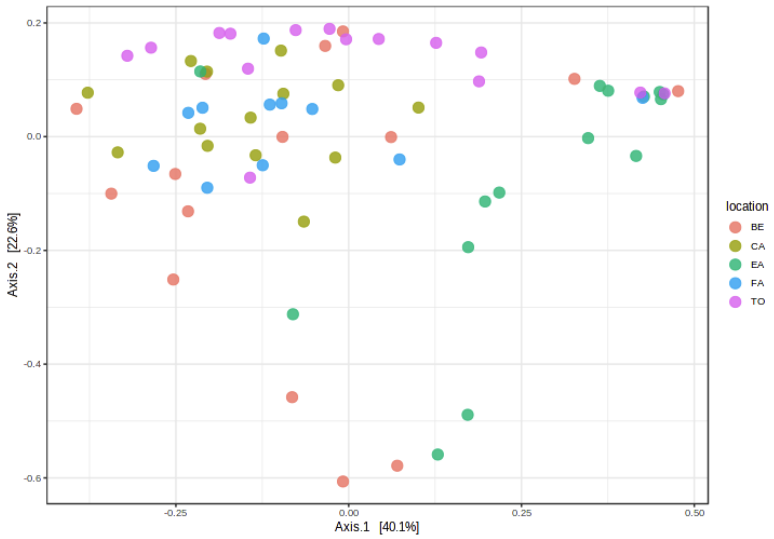


Fig 4. Visualizing feature-level beta diversity of skin microbiome using PCoA Ordination, Weighted UniFrac Distance method, and ANOSIM statistical method. (A) by location (BE = navel, CA = calves, EA = ears, FA = forearms, TO = toes); (B) by region (less = less-washed regions, more = more-washed regions).

A



B

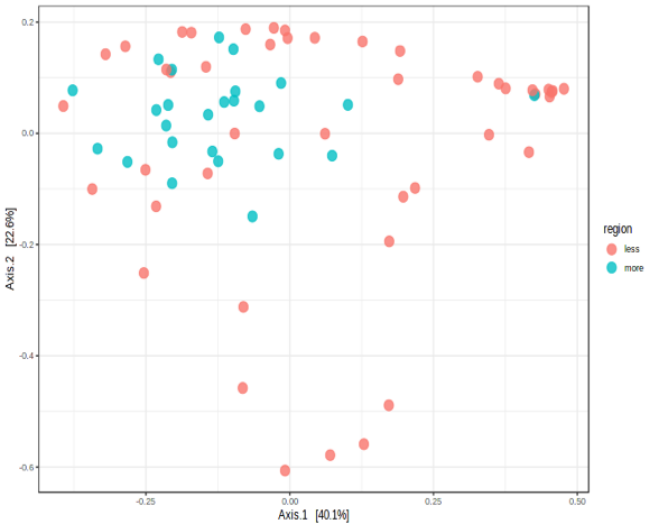


Table 1. Table showing single-factor statistical comparisons of significant differences in abundance across all five body locations using T-Test/ANOVA statistical method and 0.05 FDR-adjusted *p*-value cutoff. (A) phylum-level analysis with phyla showing significant differences in abundance highlighted; (B) genus-level analysis with genera showing significant differences in abundance highlighted.

A

	Pvalues	FDR	Statistics
Proteobacteria	3.27E-09	2.61E-08	16.106
Firmicutes	9.80E-07	3.92E-06	10.723
Actinobacteria	0.0010431	0.0027817	5.2189
Bacteroidetes	0.11788	0.23257	1.9185
Synergistetes	0.14536	0.23257	1.7712
Epsilonbacteraeota	0.31965	0.4262	1.1993
Fusobacteria	0.48411	0.55327	0.87457
Deinococcus_Thermus	0.59504	0.59504	0.69958

B

	Pvalues	FDR	Statistics
Streptococcus	7.44E-06	0.0006994	9.0141
Corynebacterium_1	3.98E-05	0.0018728	7.6705
Staphylococcus	7.33E-05	0.0022979	7.197
Escherichia_Shigella	0.00011513	0.0027056	6.8517
Lawsonella	0.00080246	0.015086	5.4088
Noviherbaspirillum	0.001692	0.026357	4.8715
Anaerococcus	0.0019627	0.026357	4.7656
Paenibacillus	0.0025074	0.028519	4.5915
Micrococcus	0.0027305	0.028519	4.5311
Bacillus	0.0034892	0.032799	4.358
Pseudomonas	0.0047755	0.038622	4.1374
Gemella	0.0049304	0.038622	4.115
Allorhizobium_Neorhi	0.0074037	0.053534	3.8312
Ezakiella	0.0081803	0.054925	3.7618
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