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Skin Microbiome – an Informational Guidance for the Personal Care Industry

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INTRODUCTION

This informational guidance serves as an introductory reference document for the United States Personal Care Product industry, on the study of Skin Microbiome and its application in the industry. Its purpose is to provide a high-level overview of this rapidly developing area. This guidance offers insights into relevant definitions, current research, and technologies pertaining to the microbiome, as well as regulatory and manufacturing concerns for cosmetic products in this space. This document does not intend to directly cover product-related claims related to skin microbiome.

There has been growing interest in the microbiome field, especially around the benefits of the skin microbiome to overall human health. The term "microbiome" was first introduced and defined in 1988 as "a characteristic microbial community occupying a reasonably well-defined habitat which has distinct physicochemical properties" [1]. The human microbiome refers to a diverse collection of microorganisms that live on the surfaces or linings of the human body, such as the skin and gut. This community includes their collective functions and roles at specific host sites. The microbiota is the collective term for all the regionalized microbial communities based on taxonomic classification. Each microbial community is uniquely specific to each person and varies depending on the location of the body. Research into the diverse and unique microbial dermal environments has demonstrated that resident microbial communities play a significant role in health and have functions yet to be discovered or fully elucidated.

A key aspect of microbiome communities is their diversity. For instance, the human skin microbiome is estimated to harbor hundreds (>600) of bacterial species [2, 3]. One of the main drivers of this diversity within the human microbiome is the "environment" of different body regions, such as the skin, oral cavity, gastrointestinal tract, urogenital tract, and respiratory tract, which each has an established subspecialty of microbes.

Abstract

The human microbiome is an inseparable part of the human body. As a result, the personal care industry is increasingly focused on the potential cosmetic benefits of addressing the microbiome. Products with microbiome-focus ingredients such as prebiotics, probiotics, and postbiotics may have a direct cosmetic benefit or may act on the skin microbiome to result in a cosmetic benefit. This informational guidance provides an introduction to the skin microbiome and its applications in the Personal Care Product Industry. It offers insights into relevant definitions, current research, technologies, and regulatory and manufacturing concerns for cosmetic products with a microbiome focus.

The skin is the body's largest organ that functions to protect and support the body with the aid of a microbiome community to protect against pathogens and environmental insults. In some instances, microorganisms are critical components of the functioning skin [4-6]. Expectedly, the personal care industry is invested in the skin microbiome.

The growing trend in developing personal care products with a microbiome focus includes prebiotics, probiotics, and postbiotics, which may also have a direct cosmetic benefit or may act on the microbiome to result in a cosmetic benefit. The human microbiome is an inseparable part of our bodies thus, product developers should consider the potential benefits and their implications of addressing the microbiome to improve skin health. The industry is in the early stages of discovering and understanding the importance of the skin microbiome and its role in skin health and personal beauty. As such, it is increasingly necessary to approach this field with a certain level of due diligence.

The overall purpose of this document is to introduce the skin microbiome, laying the foundations for further exploration in the personal care product industry.

Terminology

As the cosmetic industry leverages microbiome science to develop new products, it is essential to establish a clear and shared understanding of the microbiome, its function, and the impact of these interventions. Although several microbiome-related terms are in use today, including

prebiotic, probiotic, and postbiotic, there is currently no universally recognized glossary that defines these terms as it pertains to personal care products.

To address this gap, the International Cooperation on Cosmetic Regulations (ICCR), a voluntary international group comprised of cosmetic regulatory authorities from various countries, has developed a set of definitions for the cosmetic industry based on the scientific understanding of the skin microbiome (ICCR; www.iccr-cosmetics.org/topics-documents/14-microbiome).

Table I: List of International Cooperation on Cosmetic Regulations (ICCR) defined terms with additional insights and terminology

Microbiota	*The assemblage of microorganisms, such as bacteria, fungi, archaea, microalgae and protists present in a defined environment. This can be the human body or individual body sites, like the skin or the digestive tract.* **Although the terms microbiota and microbiome are sometimes used interchangeably, the term microbiome refers to microorganisms primarily identified by their genomic content as noted below.**
Microbiome	*A characteristic microbial community occupying a reasonably well-defined habitat which has distinct physio-chemical properties. The microbiome not only refers to the microorganisms involved but also encompasses their theatre of activity, which results in the formation of specific ecological niches. This includes their genetic material, and structural molecules, like enzymes, membrane lipids or polysaccharides.* *SKIN MICROBIOME: The skin microbiome is present on the whole skin surface, including oral cavity and mucosal surfaces of the external genital organs. The composition of the skin microbiome is dynamic, site-specific but also differs from individual to individual.* **The microorganisms found in a particular microbiome are primarily identified by analyzing their genomic material. A microorganism's genomic material determines its function or "theatre of activity" which includes its metabolic processes, the facilitators of those processes such as enzymes, and the related metabolic byproducts or metabolites.**
Probiotic	*Viable (active or dormant) microorganisms added to a cosmetic product with an intended cosmetic benefit to the host at the application site, either directly or via an effect on the host microbiome, when utilized in adequate amounts. Active microorganisms are usually defined as growing organisms, which increase their number and/or biomass. Consequently, dormant microorganisms do not grow, but keep their metabolic activity.*
Prebiotic	*Substrates (e.g., carbohydrates or other nutrients) added to a cosmetic product to be utilized by the host microbiome, with an intended cosmetic benefit to the host. Whereas pro-, post- and*
Postbiotic	*Inanimate ingredients of microbial origin added to a cosmetic product with an intended cosmetic benefit.*
Parabiotic/ Paraprobiotics	*Ingredients derived from inactivated probiotic microorganisms added to a cosmetic product with an intended cosmetic benefit. Paraprobiotics can either be inactivated microbial cells or components of cellular structures (e.g., cell walls), with or without metabolites. Based on the definition, paraprobiotics are a subgroup of postbiotics.*
Dysbiosis	*An imbalance in the resident microbial population leading to possible loss of beneficial microbial organisms, increase in harmful microorganisms, change in the overall microbial diversity and/or change in functional composition and metabolic activities.*
Synbiotic	**The combination of a probiotic and its specific prebiotic(s) that promotes the metabolism and survival of the probiotic in a target environment.**

The ICCR addresses common cosmetics safety and regulation issues and engages in constructive dialogue with relevant cosmetics industry trade associations from their respective countries (www.iccr-cosmetics.org/about-us). In the United States, the representative trade association is the Personal Care Products Council (PCPC).
The ICCR definitions provide a shared vocabulary for cosmetic industry stakeholders ***(Table I)***. To further promote the adoption of these definitions across the industry, this guidance document also provides additional insights and terminology listed in ***Table I*** (in bold).

A universally accepted set of definitions of microbiome-related terms will facilitate transparent communication amongst cosmetics industry stakeholders including manufacturers, regulators, and consumers, and lays the groundwork for future innovation.

The Skin Microbiome: Composition, Distribution, and Physiology

The skin acts as a physical barrier that protects the body from harmful environmental factors, foreign organisms, and toxic substances. On average, the skin covers an area of about two square meters (m^2), but if the skin appendages are included, the total surface area is approximately 25 m^2 [7]. This large surface has a multitude of diverse skin environments and is colonized by a wide range of microorganisms such as bacteria, archaea, fungi, viruses, and mites, most of which are harmless and beneficial to their host. The factors that drive microbial colonization of the skin by these microorganisms include skin ecology, host factors, and environmental factors. Additionally, the skin's innate and adaptive immune responses can modulate the skin microbiota, while inversely, the microbiota can also educate the immune system [5].

The human skin microbiome refers to the entire genomic collection of known or unknown microbes that reside in and on human skin. An important goal of cosmetic skin microbiome research is to understand the role microorganisms play in the well-being and homeostasis of skin. A more thorough understanding of skin microbial inhabitants, including commensals, sym-

bionts, and pathogens, provides a foundation for future research regarding the interactions between humans, microbes, and inter-microbial communities.

The skin is home to microorganisms that can be categorized as either resident or transient and are associated with the skin in either commensal, symbiotic, or pathogenic relationships. Resident microbiota are consistently found members of the skin microbiome and have a competitive advantage over the introduction of new microorganisms. These members include *Cutibacterium acnes* (formerly *Propionibacterium acnes*), *Staphylococcus epidermidis, Corynebacterium diphtheria, Corynebacterium jeikeium, Pseudomonas aeruginosa,* among other species. Whereas transient microbiota are temporary members that only colonize under conditions favorable to their survival. For example, clostridia that colonizes in the perineal area of the skin. Commensal microbiota use the material and nutrients supplied by the host without reciprocal benefits or harm to the host, such as *Staphylococcus epidermidis* Symbiotic microbiota are mutualistic for both the host and the microorganism can benefit from the association, such as *Cutibacterium*. Pathogenic microbiota, on the other hand, can cause disease, for example, *Staphylococcus aureus*. Opportunistic pathogens can cause infections under the right conditions, such as *Haemophilus ducreyi*. Genomic analysis has revealed an even greater diversity of bacteria, belonging to the phyla Actinobacteria, Firmicutes, Bacteroides, and Proteobacteria (***Figure 1***; [5]). In addition to the bacterial microbiota, there is also a fungal component associated with the skin. Like bacteria, the presence of various fungi occurs because of favorable conditions that promote their growth and persistence. Those predominately found on the skin include Malassezia spp. which are lipid-dependent yeasts. Like bacteria, Malassezia has been shown to provide a mutual benefit as skin commensals [2, 8, 9]. Other genera of fungi that have been detected on the skin include Debaromyces, Cryptococcus, and Candida [5].

The skin's surface supports distinct sets of microorganisms at different skin regions ***(Figure 1)*** that are favorable for specific microenvironments, such as dry (low humidity), moist (high humidity), or sebaceous environments. Dry skin regions have a diverse collection of microbiota including Actinobacteria, Proteobacteria, Firmicutes, and Bacteroidetes, but fewer numbers of bacteria than moist skin regions [5,10-12]. Dry skin sites include forearms, hands, legs, feet, and various parts of the face while moist skin areas include the perineal region, axillary vault, and interdigital spaces of the foot. Moist skin sites have higher humidity and temperatures and are colonized by *Staphylococcus* and *Corynebacterium* species [13].

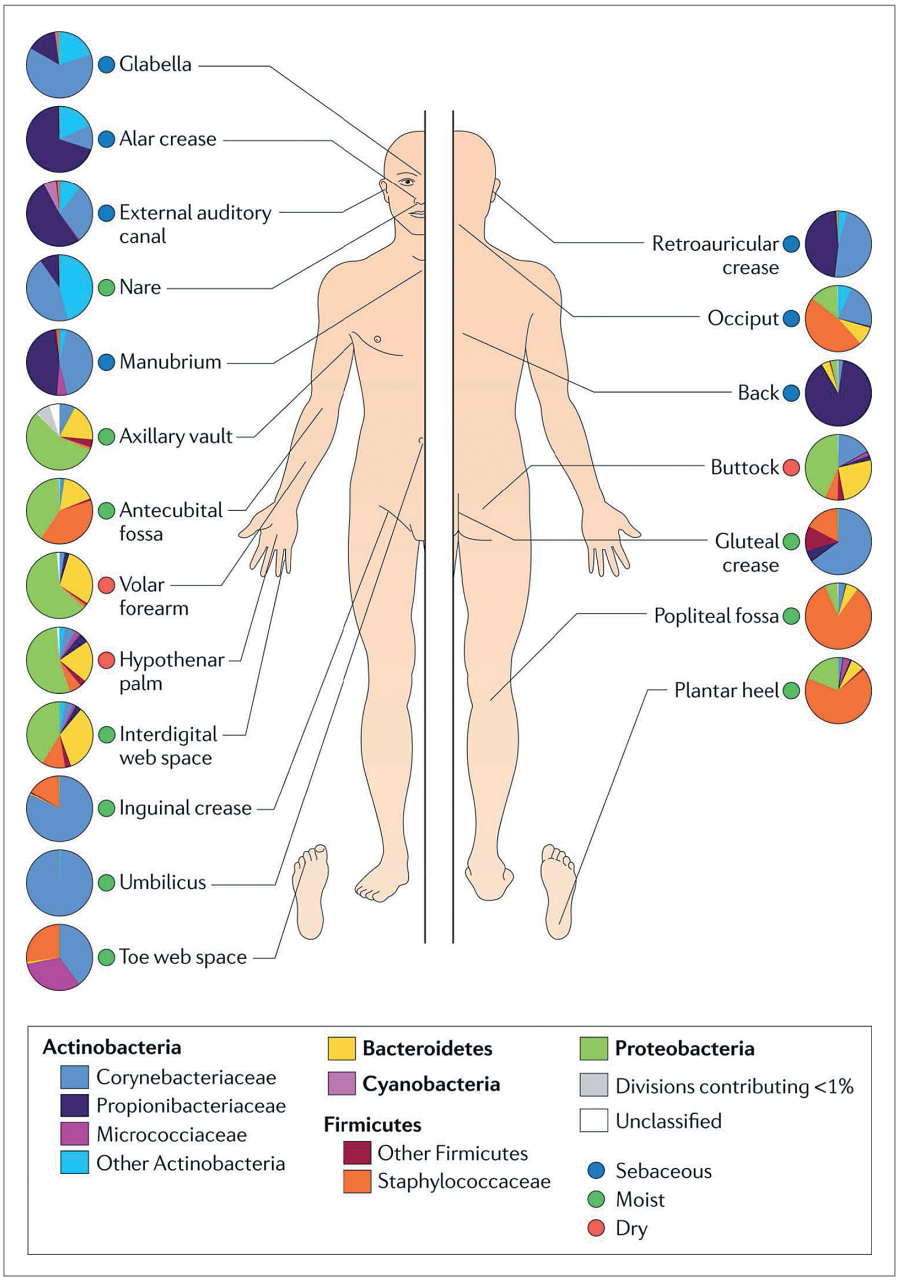

Figure 1: Distribution of bacteria on various skin sites. Adapted from source: [5].

The distribution of sebaceous glands also affects the types of microorganisms that reside at different skin sites [5]. Areas with a high density of sebaceous glands, like the face, chest, and back, encourage the growth of lipophilic microorganisms such as *Cutibacterium* and *Malassezia* species [14]. Variations in sebum production in the cutaneous environments partially account for microbial differences between biological sexes [5].

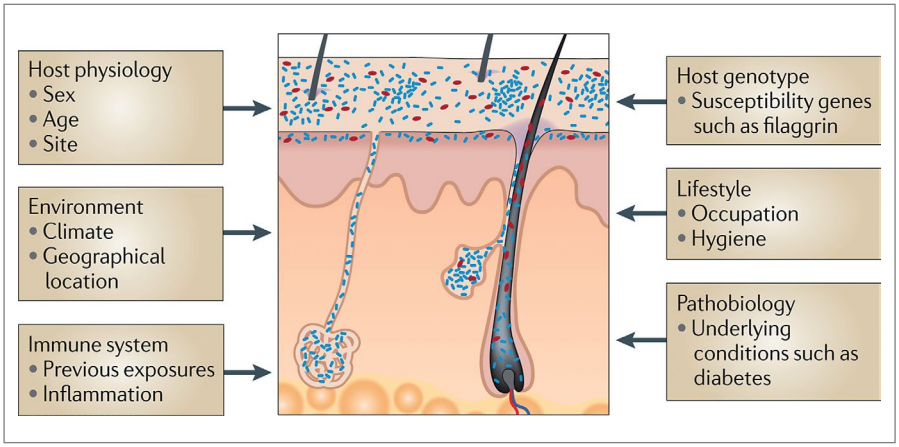

Figure 2: *Factors contributing to variation in the skin microbiome. Adapted from source [5].*

While the roles of resident microorganisms on human skin have yet to be fully understood, several endogenous and exogenous factors are known to affect the skin microbiome's composition ***(Figure 2)***. Endogenous factors, such as skin site, age, and biological sex, are specific to the host and play a role in determining the normal resident microbiota of the skin.

Advancing age during human development is an important factor that affects the skin microbiome composition at different skin sites and, thus, the colonizing microorganisms [5]. Prior to birth, research suggests that fetal skin is microbiota-free *in utero,* however, microbial colonization begins at birth, which is influenced by the method of delivery, either vaginal or cesarean section [5,15]. Further changes occur during puberty, where there is a considerable increase of *Corynebacterium* and *Cutibacterium* species and a decrease of Firmicutes, including *Staphylococcus* and *Streptococcus* species [9]. Once in adulthood, and under normal physiological conditions, the skin microbiome composition remains mostly stable despite the skin's continuous exposure to the environment [16].

Other extrinsic factors specific to the individual, such as location and environment, occupation, societal interactions, sanitary practices, and even choice of clothing, may influence the skin microbiota composition. For example, ultraviolet (UV) light from sun exposure may impact the skin microbiome; indeed, geographical locations with longitudinal and latitudinal variation in UV exposure correlate to variability in skin microbiota [5].

The use of antibiotics, cosmetics, and personal care products also contributes to the variation of the skin microbiome. However, *in vivo* research has shown that using personal care products may be possible without significant detrimental effects on the skin microbiome [17,18]. In an exploratory study by *Kromidas et al.*, a commercial personal care product designed to not impact bacterial microbiota was shown to significantly improve the recovery of face bacteria after insult with a soap, which is known to lead to bacterial cell death and injury [17].

After considering the factors that influence the composition of the skin microbiome at various skin sites, another area of interest is the mechanisms by which the skin microbiota colonizes the skin. Here, we highlight one such mechanism, the formation of biofilms. Bacterial colonization of the skin is known to occur *via* biofilms [19]. A biofilm is an aggregation of microorganisms that adhere to each other and a substrate such as the skin. The microorganisms adapt to the skin by undergoing metabolic changes, producing a gel-like matrix consisting of extracellular polymeric substances (EPS). EPS typically consist of proteins, DNA, lipids, and polysaccharides, and help the organisms attach to living or inanimate surfaces, optimize nutrient cycling, coordinate communication between cells, and protect against mechanical forces and chemical changes [20,21]. Microorganisms in biofilms often respond differently to external factors than their free-floating (planktonic) counterparts. Furthermore, microorganisms in established biofilms tend to persist and survive decontamination processes.

Biofilms are also known to have a significant impact on various skin disorders. Many skin microbiota have been studied to understand their biofilm-forming abilities and to develop strategies to inhibit biofilm production pathways to alleviate certain infections [22]. For example, biofilms have been found to play a significant role in the development of acne. *Cutibacterium acnes* reside in pores or pilosebaceous glands and can form biofilms in this environment [23]. These biofilms can have several distinct bacterial phylotypes that may cause specific outcomes [24]. Studies have shown that the IA1 phylotype of *C. acnes* recovered from individuals with acne is more efficient in early adhesion, biofilm biomass development, and significant antibiotic resistance.

The discovery that microorganisms in and on the skin can be present in biofilms also has implications for cosmetics. Biofilms on the skin can help bacteria tolerate external stressors such as exposure to UV light and pollution or various hygiene practices, including using soaps, skin abrasives, or astringents.

Microbiome Analysis

Skin microbiome studies in the personal care industry primarily focus on identifying and characterizing the microbiota present on the skin and understanding its impact on skin health. A skin microbiome study typically involves collecting microbiome samples from specific skin sites, detecting, and identifying microorganisms present in the samples, and understanding the relationship between the identified microorganisms and the skin site. This guidance document highlights the key considerations for sample collection and the sequencing tools used to identify and detect microorganisms from the collected samples.

The choice of sampling method is critical to ensure successful detection and identification of the skin microbiome of a site due to the skin's low microbial biomass [4]. Various sampling techniques are used in

skin microbiome studies, from minimally invasive skin swabs or tape-stripping to skin-destructive scrapes and biopsies. The most common technique for collecting skin microbiome samples is swabbing, which is simple to perform and causes minimal discomfort with minimal epidermal injury [25]. A pre-moistened swab is rubbed across the skin surface to collect skin microbiome samples. Numerous options are available for the swab tip material (polyester, cotton) and fiber application (spun or flocked). Additionally, a moistening solution, typically saline, is used to pre-moisten the swab before collection, and a storage and transport tube, which may also include a transport medium, is used to store the sample. These components are easy to assemble for microbiome studies and are commercially available as kits.

Tape stripping is another simple and minimally invasive sampling technique. A specially designed adhesive tape is applied to a selected section of skin with gentle and uniform pressure and then swiftly pulled from the skin [26]. Unlike swabbing, as the name implies, tape stripping removes stratum corneum cell layers and, therefore, may access deeper layers of skin. Tape striping may provide a reproducibly high amount of biomass [27]. Although tape stripping is minimally invasive, repeated application to the same section of skin in a brief period may cause transient trauma to the epidermal layers [26].

Skin biopsies are a less frequently used sampling technique for skin microbiome studies. Biopsies are used to profile the microbiome at deeper epidermal layers [28]. During the biopsy, a tool such as a skin punch, scalpel, or razor is used to remove layers of the skin at various depths. Skin biopsies provide microbiome profiles at even greater depths of the skin than tape striping. However, the invasive nature of the procedure and the potential for discomfort make it less suitable for repeated samples from the same or multiple skin sites from an individual [25].

Regardless of the method of collection, samples may be transported and stored for future processing. Swabs, for instance, are stored in a transport solution that either preserves the viability of the microorganisms (which is required for any culturing methods) or the integrity of nucleic acids (DNA, RNA) or does both. Depending on the transport solution, samples can be transported at room temperature or frozen. For storage, samples are typically stored at or below -80 °C. Additionally, a swab may be snap-frozen in its transport tube without any additional media, although this method may not preserve viability as well as a transport media or cryopreservation solution [12, 25].

Once collected, a sample must undergo several steps to identify the microorganisms present at the skin site, estimate their prevalence, and characterize their impact on the site under investigation. Today, the process primarily includes the extraction of the genomic content (most often DNA) of the sample, several steps to prepare the DNA for sequencing, sequencing the DNA, and finally bioinformatics to assign decoded DNA sequences to a microbial identity or gene function. A detailed discussion of each step in the process of analyzing the microbiome is beyond the scope of this review. Here, we highlight common strategies for identification.

The traditional benchmark for identifying microorganisms is Sanger sequencing which is now also known as first-generation sequencing [29]. As a first step, Sanger sequencing and other identification methods, such as MALDI-TOF mass spectrometry (MS), have traditionally required culturing the microorganisms in a laboratory setting. Culture-based identification methods require single isolated colonies of a microorganism to perform an identification. The Sanger-based method determines the nucleotide sequence of a single DNA template at a time [30]. An identification is made by comparing the unknown sequence to known reference sequences. MALDI-TOF MS determines identification by comparing whole-cell protein spectral profiles to reference spectra from known species and strains. Most microorganisms, however, are unculturable [31], which limits the discovery of the full spectrum of microbial diversity within a skin microbiome community using culture-based identification methods.

Two significant advancements have revolutionized the study of the microbiome. The first was the development of high-throughput sequencing (HTS) or next-generation sequencing (NGS) technologies, also known as second-generation sequencers [30]. The second was the creation of the Human Microbiome Project Consortium [32]. These advancements have made microbiome analysis primarily culture-free and have introduced a new tool in microbiome studies called metagenomics.

NGS allows for the high-throughput sequencing of the entire genomic content of a microbial community in a sample. It removes the need for an isolation and culture step, which makes it possible to detect microorganisms that cannot be cultured in a lab. Second-generation sequencers decode millions of short DNA sequences (<450 bp) per run. Currently, most second-generation sequencers available for commercial use rely on a sequencing method called sequence by synthesis (SBS). The fundamental principle behind SBS involves fixing billions of DNA fragments to a solid support, converting double-stranded DNA into single-stranded templates, attaching a primer, incorporating complementary nucleotides *via* DNA polymerase to a new DNA strand, and detecting this incorporation. Instruments mainly vary based on their sequence detection method, including fluorescence imaging from fluorescently labeled nucleotides or measuring changes in pH [30, 33]. Third- and fourth-generation sequencers are also now available that sequence longer reads (up to 1 Mb) in a shorter amount of time and utilize different chemistries to detect the sequence reaction [33, 34]. NGS has significantly reduced the time and cost to sequence entire genomes. While the Human Genome Project took 15 years to complete 92% of the human genome, today, with NGS, this can be accomplished in a day. (www.genome.gov/about-genomics/educational-resources/fact-sheets/human-genome-project).

The second advancement, the Human Microbiome Project Consortium (HMP), was an NIH initiative launched in 2007. Deploying NGS, the HMP successfully characterized the microbiome of three

hundred (300) healthy individuals across various body sites and gained insights into the functions of the human microbiome [32]. The project facilitated the development of metagenomic studies and bioinformatic analysis of microbial sequence data (hmpdacc.org/hmp/).

In skin microbiome studies, there are two sequencing approaches: targeted or amplicon sequencing and whole metagenomic or shotgun metagenomic sequencing. In amplicon sequencing, only a single marker gene or region of a gene is sequenced. The targeted gene or region is isolated from the remainder of the genome through polymerase chain reaction (PCR) amplification. The 16S ribosomal RNA (rRNA) gene, found only in bacteria, is used to identify bacteria, while the internal transcribed spacer (ITS) region between the fungal rRNA genes is used to identify fungi [4]. The 16S rRNA gene has evolutionarily conserved regions present in all bacteria and nine variable elements specific to individual species. When the 16S rRNA gene of a bacterium is fully sequenced, the bacterium can often be taxonomically classified to the species level [35, 36]. In amplicon sequencing to identify bacteria, only a portion of the 16S rRNA gene, covering a subset of the variable regions, is sequenced. In skin microbiome studies, the preferred region includes variable region 1 (V1) to variable region (V3). In amplicon sequencing to identify fungi, the preferred targeted region includes the internal transcribed spacer regions 1 or 2 (ITS1, ITS2) [4]. Once amplified, the select portions of rRNA genes or gene regions (16S rRNA for bacteria, ITS for fungi) are sequenced using one of the available NGS technologies.

The sequence data generated (reads) are analyzed to determine the identity of the microorganisms present in a sample. Various computational tools are available that automate the process. The process involves quality control steps to remove erroneous or non-relevant reads (low-confidence nucleotide or base calls, primer and adaptor sequences, untargeted eukaryotic DNA sequences, etc.) followed by assigning a taxonomic classification to accepted reads to determine the microorganisms present in a sample (taxonomic assignment). There are currently two methods for taxonomic assignment: clustering similar sequences with >97% similarity into operational taxonomic units (OTU) or grouping identical (100% match) sequences using the amplicon sequencing variants (ASV) method. The OTU clustering method is more widely used and assigns taxonomic classification by either de novo approaches, which attempts to classify previously unencountered sequences, or reference-based assignment in which the OTU sequence is compared to sequences of known taxa in a reference database. OTU assignment typically yields genus-level identification. In contrast, ASV takes an exact sequence read and the frequency at which it occurs in the sequencing dataset to determine the probability that the read is not due to a sequence error. Only reads with high statistical confidence are compared to a reference database to provide identification at the species level [37].

In shotgun or whole metagenomic sequencing (wMGS), all the genomic content (and not just a single gene or region) present in a sample is sequenced. Shotgun sequencing provides a comprehensive profile of the microbial community at a site and insights into their function.

Second-generation sequencers are routinely used in wMGS, which requires the microbiome genomic DNA to be fragmented into short DNA fragments. Similar to amplicon sequencing, in wMGS, the generated sequence reads are preprocessed to remove unwanted reads before taxonomic classification, which is accomplished through assembly-based or assembly-free methods [38].

In assembly-based methods, overlapping regions in sequence reads are pieced together to produce a consensus contiguous sequence called a contig. A wMGS dataset typically contains multiple contigs belonging to the original genomes of the different microorganisms. Related contigs of a single microorganism are arranged onto a scaffold that includes gaps of unresolved sequences to reconstruct the original genome. This scaffold is clustered or binned into a taxonomy group, such as a species [38].

In assembly-free methods, reads are directly mapped to genomes in reference databases. Assembly-free methods also include mapping reads to discriminative marker genes from reference sequences. Both assembly and assembly-free methods have several computational programs, each with its own advantages and drawbacks, that perform the steps necessary to yield a taxonomic assignment [38].

In addition to identifying microorganisms present in a sample, NGS techniques also estimate the prevalence of identified microorganisms. It is important to note that no NGS technique provides absolute quantification of microbial species in a sample. Instead, the number of reads assigned to each identified microorganism determines the relative abundance of microorganisms in the sample. The relative abundance represents the proportions of microorganisms in a sample, reflecting how common or rare a microorganism is relative to other microorganisms [39]. The relative abundance represents normalized values and the fraction of the organism observed relative to the sum of all observed organisms [40].

Measures of diversity are also determined when evaluating the skin microbiome. Alpha diversity and beta diversity are the two most used diversity metrics. Alpha diversity measures the biodiversity within a sample and includes richness and evenness ***(Figure 3)***. Richness is the sum of observed features (OTUs, genus, species, genes) in the sample while evenness measures the proportion or distribution of each observed feature within the sample. Statistical indices used to measure alpha diversity include Chao 1, Shannon, and Simpson indices. The Chao 1 index incorporates only richness, while the Shannon and Simpson indices consider richness and evenness (reviewed in [41]).

Beta diversity describes the diversity among samples within a study ***(Figure 4)***. The two most used methods are Bray Curtis and UniFrac (unweighted and weighted). Bray Curtis compares the presence and frequency of an observed taxon shared among samples. Unweighted UniFrac incorporates the phylogenetic diversity between samples and

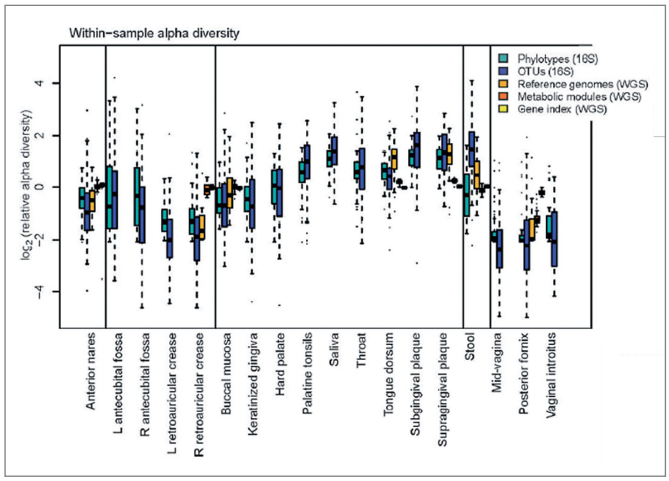

Figure 3: Example alpha diversity plot adapted from: Human Microbiome Project Consortium. Structure, function, and diversity of the healthy human microbiome [32].

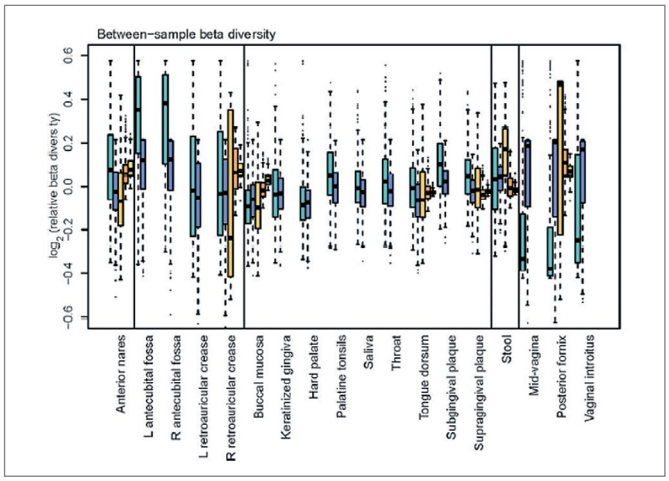

Figure 4: Example beta diversity plot adapted from: Human Microbiome Project Consortium. Structure, function, and diversity of the healthy human microbiome [32].

focuses only on richness, while weighted UniFrac incorporates both richness and evenness (reviewed in [41]).

NGS remains the tool of choice to survey the microbiome, detailing the identity, relative abundance, and diversity of microorganisms in skin samples. Increasingly, microbiome studies are focused on determining the role or function of skin microorganisms in skin health. Shotgun metagenomic sequencing provides some insight into the functional profile of the microbial community by analyzing all genes in a sample. To gain deeper insights into function, techniques such as metatranscriptomics, which profiles genes that are expressed, and metabolomics, which profiles the metabolites present in a sample, can be used [42]. One way to better understand the function of microorganisms is to have a culture of those microorganisms to conduct phenotypic analyses. This has led to the development of culturomics, which creates high throughput culturing conditions to identify the conditions needed to grow microbial community members, especially previously unknown members, identified in metagenomics [43].

Safety and Regulatory Considerations

As with any other cosmetic and personal care product, cosmetic products with prebiotic, probiotic, or postbiotic ingredients must be safe for consumer use throughout their entire life cycle. Each ingredient type presents unique safety and regulatory challenges; some challenges will significantly differ from those of current non-microbiome-related ingredients. Safety should be established as currently practiced for other cosmetics while introducing new safeguards where necessary. This may include expert development, validation, and application of new test methods.

Prebiotic or postbiotic ingredients are not fundamentally different from any other cosmetic ingredient. In fact, many prebiotic ingredients, such as sugars, amino acids, vitamins, and minerals, are already commonly used as cosmetic ingredients. Postbiotic ingredients are protein-based materials, which are also a well-established ingredient in the industry.

On the other hand, probiotic ingredients and cosmetics pose a more significant challenge to evaluate for safety, produce, and market. Probiotic cosmetics will contain viable (active or dormant) microorganisms, which may exceed established microbial limits for cosmetics. According to the FDA's Bacteriological Analytical Manual (BAM) and ISO Standard 17516:2014, the acceptable limits for total non-pathogenic microorganisms in cosmetics are 100 CFU/g or mL in eye area products and 1000 CFU/g or mL for other products. Furthermore, the US Food, Drug, and Cosmetic Act (FD&C) considers a cosmetic product to be contaminated or adulterated ''if it contains any poisonous or deleterious substance which may render it injurious to users under the conditions of use prescribed in the labeling thereof, or under such conditions of use as are customary or usual…''. Microbial contamination that is injurious to health is considered a ''poisonous or deleterious substance''. Therefore, a probiotic or any other microbial-based ingredient that is proven safe, non-pathogenic, and within the established microbial limits would be considered uncontaminated or unadulterated.

To ensure the safety of probiotic products, manufacturers should ensure the formulated microorganism strain is free of any contaminating strains, especially pathogens, and establish the product's use-life during which the strain maintains its characteristics. If needed, the manufacturer should also address required storage conditions and consider detailed and clear use instructions and any significant cautions on the product's label.

Manufacturers of probiotic cosmetics must ensure and justify the safety of their products and demonstrate that the added probiotic strains are not acting as inoculation. Additionally, preserving cosmetic products with probiotics will be challenging as our current standard preservative systems will likely render strains inert. To overcome the challenge of preserving probiotic cosmetics, formulators, and microbiologists will need to develop innovative solutions. This may involve using a combination of gentler preservative systems, as well as adjusting pH levels or water activity. Packaging solutions

such as lyophilization, single-use containers, airless pumps, bag-on-valve packaging, manufacturing processes like aseptic processing, and proper storage conditions like refrigeration, can also play a vital role in preserving the quality and safety of probiotic cosmetics. It is worth noting that selective prebiotic substances may enhance the potential for contamination in the manufacturing and production environment. Therefore, tighter standards in storing and compounding these substances may be required to avoid contamination.

Currently, no global regulations or requirements specifically address microbiome-focused cosmetics or cosmetic ingredients intended to provide a cosmetic benefit. As discussed above, the ICCR's Microbiome and Cosmetics taskforce represents an international effort to increase understanding of cosmetics products that focus on the skin microbiome. In the United States, the FDA will categorize skin microbiome products as either cosmetics, drugs, or other categories based on their overall positioning or claim. The FDA defines a cosmetic as "a product (excluding pure soap) intended to be applied to the human body for cleansing, beautifying, promoting attractiveness, or altering the appearance". A drug is any product "intended for use in the diagnosis, cure, mitigation, treatment or prevention of disease" or any product "intended to affect the structure or any function of the body of human or other animals" (FD&C Act, 201 (g) (1)).

Manufacturers may face legal, regulatory, and public relations challenges if they use unsubstantiated or vague skin microbiome-related claims. Companies' marketing, regulatory, and legal entities must work together to ensure the validity of promoting their microbiome-themed products to consumers, which is crucial in building consumer trust in science. Understanding the regulatory framework early in marketing these products can minimize commercial risk and ensure regulatory compliance. It is important to keep in mind that the recent enactment of the Modernization of Cosmetics Regulations Act of 2022 (MoCRA) indicates that governmental review may increase in the future.

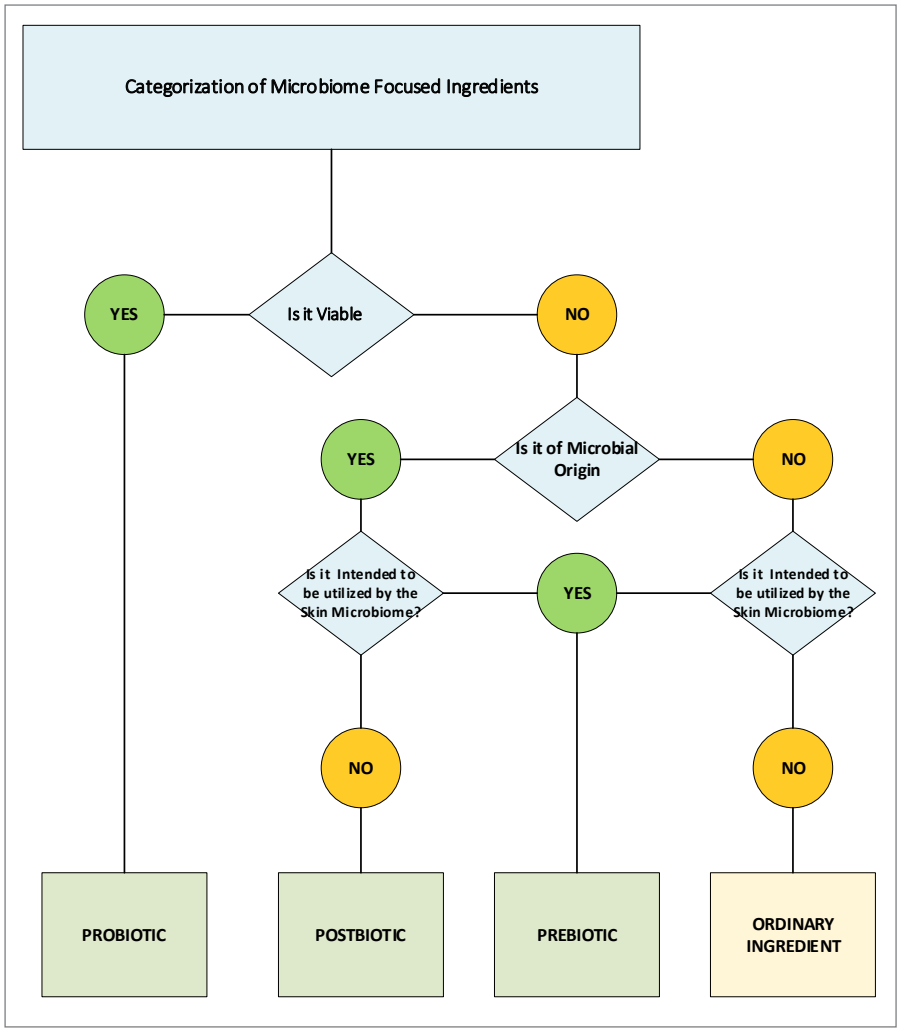

Figure 5: Schematic to determine if an ingredient is a prebiotic, probiotic, postbiotic, or just an ordinary cosmetic ingredient. (See definitions in ***Table I****). For ingredient examples that may be pre-, pro-, and postbiotic refer to* ***Table II****.*

DISCUSSION AND CONCLUSION

Research has shown that the diverse and unique microbial communities constituting the human microbiome play a significant role in health. The skin and its microbial community protect and support the human body, protecting against pathogens and environmental damage. As the importance of the microbiome to human health is increasingly recognized, this informational guidance is intended as an introductory reference to facilitate further exploration of the potential benefits of microbiome-based ingredients and products on the skin. Personal care or cosmetics products can be designed to maintain the skin microbiome by incorporating prebiotics, probiotics, or postbiotics ingredients.

In addition to claim substantiation, the regulation of microbiome technologies and products will be partly driven by a material's ingredient or product categories, which fall into those described by the flowchart below ***(Figure 5)***. Those materials composed of live microorganisms (i.e., probiotic category) may also be considered in a separate category with biologically sourced products. Some examples of prebiotic, probiotic, and postbiotic ingredients are shown in ***Table II*** below.

Even as this guideline provides high-level insight into the rapidly evolving market by defining terminology and currently available technologies for products that enhance or modify the skin microbiome, it raises awareness for future regulatory and manufacturing concerns for these cosmetic products.

Table II: Example Ingredient Types. The skincare market has embraced distinct approaches, targeting the skin microbiome on three different fronts in the market and using multiple types of ingredients or strategies. Examples are listed below.

Ingredient Type	Examples
Probiotics	○ Probiotics of lactic acid-forming bacteria like *Lactobacillus*, non-lactic acid forming bacteria like *Staphylococcus epidermidis*, and yeast such as *Saccharomyces.* ○ Proprietary mixtures (viable or likely viable): e.g., a preparation containing *Bacillus coagulans*, a mixture of *Lactobacillus acidophilus* and *Bifidobacterium bifidum.* ○ Viable undefined microorganisms such as yogurt or yogurt powder that were not heat-inactivated.
Prebiotic	○ Carbohydrates: Alpha-glucan oligosaccharide, Fructooligosaccharides (FOS), Fructose, Inulin, Fiber, Beta-glucans, Maltodextrin, Mannose, Inositol, Galactoarabinan. ○ Plant- or Algae-derived ingredients: *Avena sativa* (oat) kernel extract/flour/oil, *Viola tricolor* (wild pansy) extract, *Cocos nucifera* (coconut tree) extract, *Salvia hispanica* (chia) extract, Allantoin, *Polymnia sonchifolia* (yacón daisy) root juice, *Cyathea cumingii* (fern) leaf extract, *Morinda citrifolia* callus (noni) culture lysate, *Chlorella vulgaris* extract, *Parachlorella beijerinckii* exopolysaccharides (alguronic acid), *Kappaphycus alvarezii* extract, etc. ○ Vitamins or pro-vitamins: Tocopherol, Niacinamide, Panthenol. ○ Postbiotic derivatives: Rhamnose-rich polysaccharide, Ectoin, Saccharomyces/rice ferment filtrate, *Pseudoalteromonas* exopolysaccharides. ○ Amino acids and peptide derivatives: Hydrolyzed yeast protein, Glutamic acid. ○ Organic acids: Lactic acid, Citric acid. ○ Minerals and metals: Selenium, Oligo-elements, Strontium.
Postbiotic	○ Ferments, lysates, extracts, filtrates, or any combination of these ingredients that have been obtained by means of probiotic bacteria (*Bacillus*, *Bifidobacterium*, *Lactobacillus*, *Lactococcus*, *Vitroscilla*, *Streptococcus thermophilus*, *Leuconostoc*) or fungi used primarily as fermentation facilitators (*Saccharomyces, Candida bombicola, Kloeckera, Hansenula-Pichia,* and *Aspergillus*). ○ Non-viable microorganisms (inactivated/heat-killed), mostly lactic-acid forming bacteria: *Enterococcus faecalis*, *Lactobacillus* (*paracasei, casei, acidophilus*), *Lactococcus*, or *Vitroscilla* filiform. Non-viable microorganisms may also have a prebiotic effect. ○ Metabolic products/by-products (isolated): bacteriocin extract, endolysins, Ectoin, succinic acid, lactic acid, sodium hyaluronate, and milk proteins. ○ Biofermentes: Microbial products obtained via enzymatic actions, typically by bacteria and yeast, which break large, complex molecules into smaller, simpler ones. ○ Filtrate: Bioferment filtrates are another variation of bioferments. Most are water-soluble and hence, can be readily incorporated into a wide range of skin and hair care formulations. ○ Cell lysate: Bacterial lysates are produced by inducing stress and rupturing the bacterial cell, rendering it non-viable. The ruptured cell then releases its valuable intracellular components, providing the benefits of the live bacteria without the live bacteria.

Manufacturers of cosmetics and personal care products are aware that depending on the product category and claims, the regulation of these products can differ. Some may be regulated as cosmetics, and some as drugs. Therefore, the functions of products that modify or enhance the skin microbiome should be well-defined.

Products that support the microbiome by adding a prebiotic or postbiotic will perhaps have simpler designs and fewer hurdles than products containing live probiotic organisms. Some questions have been raised regarding probiotics in cosmetics, and many of these questions still need to be answered. For example, many global regulatory agencies and pharmacopeia, such as the FDA, EMA, USP, and Ph. Eur., require the characterization of the microbial profile (bioburden) of products and documentation of the efficacy of preservatives. If the product is designed to contain a live organism, traditional microbial limits, and bioburden assays will not be appropriate or sufficient. The NGS technologies discussed herein may offer the solution for necessary testing, and an additional validation burden for this alternative test would need to be assumed. Likewise, evaluating the use and necessity of preservatives is essential to determine if they are needed in the case of probiotic-based products and if they are detrimental to the product and claims of safety or efficacy. The extent of these safety or efficacy claims will also determine how a product is regulated, whether as a cosmetic or a drug. In addition to the consideration of safety for the consumer, there are considerations such as shelf life and storage constraints for these products, the impact on the live organisms, or even the presence of spores, and the critical quality attributes of the products.

It is becoming increasingly common to find personal care and cosmetic products that contain prebiotics, probiotics, or postbiotics in the market. However, the use of such products can have both intentional and unintentional effects on the skin microbiome. Given that the microbiome plays a crucial role in maintaining healthy body functions and that it persists throughout our lifetime, it is important to exercise diligence when developing microbiome-focused personal care products.

Conflict of Interest Statement

The authors of this paper are members of the Microbiology Committee and Microbiome Task Force of the Personal Care Products Council (PCPC). The viewpoints

expressed in this paper are solely those of the authors and do not necessarily reflect those of any Competent Authority, PCPC, or our respective companies.

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References

[1] *Whipps J.M., Lewis K., Cooke R.C.,* Mycoparasitism and Plant Disease Control, in Fungi in Biological Control Systems, *M.N. Burge,* Editor, Manchester University Press: Manchester, UK, 1988, 161-187.

[2] *Li H.,* The Human Skin Microbiome in Health and Skin Diseases, in Metagenomics of the Human Body, K.E. Nelson, Editor, Springer New York: New York, NY., 2011, 145-163.

[3] *Saheb Kashaf S., Proctor D.M., Deming C., Saary P., Hölzer M.,* NISC Comparative Sequencing Program, Taylor M.E., Kong H.H., Segre J.A., Almeida A., Finn R.D. , Integrating cultivation and metagenomics for a multi-kingdom view of skin microbiome diversity and functions, Nat Microbiol, **7** (2022) 169-179.

[4] *Byrd A.L., Belkaid Y., Segre J.A.,* The human skin microbiome, Nat Rev Microbiol, **16** (2018) 143-155.

[5] *Grice E.A., Segre J.A.,* The skin microbiome, Nat Rev Microbiol, **9** (2011) 244-253.

[6] *Kumar A., Chordia N.,* Role of Microbes in Human Health Appli Microbiol Open Access, **3** (2017) 1-3.

[7] *Boxberger M., Cenizo V., Cassir N., La Scola B.,* Challenges in exploring and manipulating the human skin microbiome, Microbiome, **9** (2021) 125.

[8] *Wu G, Zhao H, Li C, Rajapakse MP, Wong WC, Xu J, Saunders CW, Reeder NL, Reilman RA, Scheynius A, Sun S, Billmyre BR, Li W, Averette AF, Mieczkowski P, Heitman J, Theelen B, Schröder MS, De Sessions PF, Butler G, Maurer-Stroh S, Boekhout T, Nagarajan N, Dawson TL Jr.,* Genus-Wide Comparative Genomics of Malassezia Delineates Its Phylogeny, Physiology, and Niche Adaptation on Human Skin. PLoS Genet, **11** (2015), e1005614.

[9] *Oh J., Conlan S., Polley E.C., Segre J.A., Kong H.H.,* Shifts in human skin and nares microbiota of healthy children and adults, Genome Med, **4** (2012) 77.

[10] *Costello E.K., Lauber C.L., Hamady M., Fierer N., Gordon J.I., Knight R.,* Bacterial community variation in human body habitats across space and time, Science, **326** (2009) 1694-1697.

[11] *Gao Z., Tseng CH., Pei Z., Blaser M.J.,* Molecular analysis of human forearm superficial skin bacterial biota. Proc Natl Acad Sci U S A, **104** (2007) 2927-2932.

[12] *Grice E.A., Kong H.H., Conlan S., Deming C.B., Davis J., Young A.C,* NISC Comparative Sequencing Program, *Bouffard G.G., Blakesley R..W, Murray P.R., Green E.D., Turner M.L., Segre J.A.,* Topographical and temporal diversity of the human skin microbiome, Science, **324** (2009) 1190-1192.

[13] *Marples, M.,* The Ecology of the Human Skin, A seminal and comprehensive work of classical dermatological microbiology, ed. C.C. Thomas, Springfield, Illinois: Bannerstone House, 1965.

[14] *Roth R.R., James W.D.,* Microbial ecology of the skin, Annu Rev Microbiol, **42** (1988) 441-464.

[15] *Chu D., Ma J., Prince A., Antony K., Seferovic M., Aagaard K.,* Maturation of the infant microbiome community structure and function across multiple body sites and in relation to mode of delivery, Nat Med, **23** (2017) 314-326.

[16] *Oh J., Byrd AL., Park M.,* NISC Comparative Sequencing Program, *Kong H.H., Segre J.A.,* Temporal Stability of the Human Skin Microbiome, Cell, **165** (2016) 854-866.

[17] *Kromidas L., Goins K., Lapczynski A., Kromidas G., Weiss C.R.,* A One-Day Microbiome-Friendly Study, HAPPI, **59** (2022) 48-56.

[18] *Murphy B., Hoptroff M., Arnold D., Eccles R., Campbell-Lee S., In vivo* impact of common cosmetic preservative systems in full formulation on the skin microbiome, PLoS One, **16** (2021) e0254172.

[19] Flemming H.-C., Wuertz S., Bacteria and archaea on Earth and their abundance in biofilms, Nature Reviews Microbiology, **17** (2019) 247-260.

[20] *Costerton J.W., Lewandowski Z., Caldwell D.E, Korber D.R., Lappin-Scott H.M.,* Microbial biofilms, Annu Rev Microbiol, **49** (1995) 711-45.

[21] *Donlan R.M.,* Biofilms: microbial life on surfaces, Emerg Infect Dis, **8** (2002) 881-890.

[22] *Brandwein, M., Steinberg D., Meshner S.,* Microbial biofilms and the human skin microbiome. NPJ Biofilms Microbiomes, **2** (2016) 3.

[23] *Coenye T., Honraet K., Rossel B., Nelis H.J.,* Biofilms in skin infections: *Propionibacterium acnes* and *acne vulgaris*, Infect Disord Drug Targets, **8** (2008)156-159.

[24] *Platsidaki, E., Dessinioti C.,* Recent advances in understanding *Propionibacterium acnes (Cutibacterium acnes)* in acne, **7** (2018) F1000Res.

[25] *Kong H.H., Andersson B., Clavel T., Common J.E., Jackson S.A., Olson N.D., Segre J.A., Traidl-Hoffmann C.,* Performing Skin Microbiome Research: A Method to the Madness, Journal of Investigative Dermatology, **137** (2017) 561-568.

[26] *Barber, C., Boiko S.,* Tape stripping: investigational, diagnostic, and therapeutic uses in dermatology, Clin Dermatol, **40** (2022) 355-362.

[27] *Chng K.R., Tay A., Li Ch., Ng A., Wang J., Suri B., Matta S., Mcgovern N., Janela*

B., Wong X.F., Sio Y., Au B., Wilm A., Sessions P., Lim Th., Tang M., Ginhoux F., Connolly J., Lane E., Nagarajan N., Whole metagenome profiling reveals skin microbiome-dependent susceptibility to atopic dermatitis flare, Nat Microbiol, 2016. **1** (2016) 16106.

[28] *Grice E.A., Kong H.H., Renaud G., Young A.C., NISC Comparative Sequencing Program, Bouffard G.G., Blakesley R.W., Wolfsberg T.G., Turner M.L., Segre J.A.,* A diversity profile of the human skin microbiota, Genome Res, **18** (2008) 1043-1050.

[29] *Sanger F., Nicklen S., Coulson A.R.,* DNA sequencing with chain-terminating inhibitors, Proc Natl Acad Sci U S A, **74** (1977) 5463-5467.

[30] *Heather J.M., Chain B.,* The sequence of sequencers: The history of sequencing DNA, Genomics, **107** (2016) 1-8.

[31] *Lagier J.C., Edouard S., Pagnier I., Mediannikov O., Drancourt M., Raoult D.,* Current and past strategies for bacterial culture in clinical microbiology, Clin Microbiol Rev, **28** (2015) 208-236.

[32] Human Microbiome Project Consortium, Structure, function and diversity of the healthy human microbiome, Nature, **486** (2012) 207-214.

[33] *Pervez M.T., Hasnain M.J.U., Abbas S.H., Moustafa M.F., Aslam N., Shah S.S.M.,* A Comprehensive Review of Performance of Next-Generation Sequencing Platforms, Biomed Res Int, **2022** (2022) 3457806.

[34] *van Dijk E.L., Jaszczyszyn Y., Naquin D., Thermes C.,* The Third Revolution in Sequencing Technology, Trends Genet, **34** (2018) 666-681.

[35] *Wayne L.G., Brenner D.J., Colwell R., Grimont P., Krichevsky M., Moore L.H., Moore W.E.C., Murray R.G.E., Stackebrandt E., Starr M.P., Truper H.G.,* Report of the Ad Hoc Committee on Reconciliation of Approaches to Bacterial Systematics, International Journal of Systematic and Evolutionary Microbiology, **37** (1987) 463-464.

[36] *Woese C.R., Fox G.E.,* Phylogenetic structure of the prokaryotic domain: the primary kingdoms, Proc Natl Acad Sci U S A, **74** (1977) 5088-5090.

[37] *Callahan B.J., Wong J., Heiner C., Oh S., Theriot C.M., Gulati A.S., McGill S.K., Dougherty M.K.,* High-throughput amplicon sequencing of the full-length 16S rRNA gene with single-nucleotide resolution, Nucleic Acids Research, **47** (2019) e103-e103.

[38] *Quince C., Walker A.W., Simpson J.T., Loman N.J., Segata N.,* Shotgun metagenomics, from sampling to analysis. Nat Biotechnol, **35** (2017) 833-844.

[39] *Gloor G.B., Macklaim J.M., Pawlowsky-Glahn V., Egozcue J.J.,* Microbiome Datasets Are Compositional: And This Is Not Optional, Frontiers in Microbiology, **8** (2017) 2224.

[40] *Lin H., Peddada S.D.,* Analysis of microbial compositions: a review of normalization and differential abundance analysis, npj Biofilms and Microbiomes, **6** (2020) 60.

[41] *Mbareche H., Dumont-Leblond N., Bilodeau G.J., Duchaine C.,* An Overview of Bioinformatics Tools for DNA Meta-Barcoding Analysis of Microbial Communities of Bioaerosols: Digest for Microbiologists, Life (Basel), **10** (2020) 185.

[42] *Aguiar-Pulido V., Huang W., Suarez-Ulloa V., Cickovski T., Mathee K., Narasimhan G.,* Metagenomics, Metatranscriptomics, and Metabolomics Approaches for Microbiome Analysis: Supplementary Issue: Bioinformatics Methods and Applications for Big Metagenomics Data, Evolutionary Bioinformatics, **12** (2016) Suppl1. 5-16, EBO.S36436.

[43] *Diakite A., Dubourg G., Dione N., Afouda P., Bellali S., Ngom I.I., Valles C., lamine Tall M., Lagier J.C., Raoult D.,* Optimization and standardization of the culturomics technique for human microbiome exploration, Scientific Reports, **10** (2020) 9674.

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