

The Microbiome of Healthy Skin

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Abstract

Over the last decade, radical advances in sequencing technologies have provided the tools with which to characterize microbial communities with unprecedented completeness and the consequent adoption of the term microbiome to describe the totality of microorganisms associated with a particular ecological niche. The application of these techniques has driven a renaissance in microbiology and nowhere is this truer than in our rapidly advancing understanding of the human-associated microbiome in all its complexity.

The work of the Human Microbiome Project and numerous other research groups has led to characterization of the skin microbiome in healthy and pathological skin, across body sites and populations. The emerging picture is one of a holistic association between skin and microbiome where healthy skin is the foundation of a balanced microbiome and where a balanced microbiome contributes to maintenance of healthy skin.

Keywords: Antimicrobial lipid, antimicrobial peptide, commensal microbe, microbiome, pathogen

1.1 Introduction

1.1.1 Retrospective

From the 1950s, pioneering microbiology studies began to reveal more about the composition of microbes on human skin. During this time much was

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learned regarding the identity of the dominant skin resident microorganisms under normal conditions and their association with disease. Typically, skin resident microorganisms are classified as those whose lifecycles are near permanently wedded to the skin (often referred to as skin resident or skin commensal microorganisms) and those which use the skin as a temporary conduit or transport mechanism by which to complete an aspect of their life cycle (the transient microbial population; for example, the role of hands as vectors for fecal or oral transmission of enteropathogenic *Escherichia coli*).

As the title of this chapter suggests, the focus will be on those resident or commensal microorganisms for which skin is their permanent home. These microorganisms derive their nutrients from skin, such as skin and sebaceous lipids or from other community members and the skin microenvironment determines local ecology and growth rate and limitation.

As will be discussed in more detail later, the ever-increasing accessibility of next generation sequencing techniques and their application to the field of microbiology continues to transform our understanding of the skin microbiome at a taxonomic and functional level. As this understanding grows, so does the need to embed those insights in an understanding of how local skin conditions (nutritional, microenvironmental, physical, chemical and immunological) impact the local microbial ecology, which may vary from the centimeter scale of occluded, non-occluded, sebaceous or non-sebaceous, hair or non-hairy body sites to the micron length scales of an individual hair follicle, eccrine gland or skin squame.

Pioneering work in the 1960s by Donald Pillsbury and Mary Marples laid essential groundwork for our understanding of how ecological constraints, such as the fundamental aridity of skin, affects what skin microorganisms. Later, work was done on the importance of skin lipids as nutrient sources and as natural antimicrobials [1–3]. This work helped to ground our understanding of how the normal processes of healthy skin modulates its microbiome by maintaining its local environment within narrow windows of pH, sebaceous activity, aridity, osmolarity and desquamation and how differences in these parameters help to explain the normally occurring differences in the microbiome between body sites [4–7].

That this is a two-way relationship, with microbes impacting skin condition and vice versa, was confirmed through seminal investigations by Roger Marples, Mary Stewart and others. These authors demonstrated how commensal skin microorganisms contribute to the normal functioning of healthy skin through the hydrolysis of sebaceous triglycerides into free fatty acids and glycerol, thereby contributing to the maintenance of normal skin acidity and hydration [8–13]. Such insights into the relationship between human lipids, their role as microbial nutrients and the impact on microbial

localization to skin invaginations, such as hair follicles, were confirmed in light microscopy work by Montes [14]. More recently, the application of fluorescence *in-situ* hybridization (FISH) [15, 16] and cryosectioning scanning electron microscopy (SEM) techniques [17] have provided researchers with an unprecedented ability to visualize the spatial localization of micro-organisms at the micron scale (Figure 1.1).

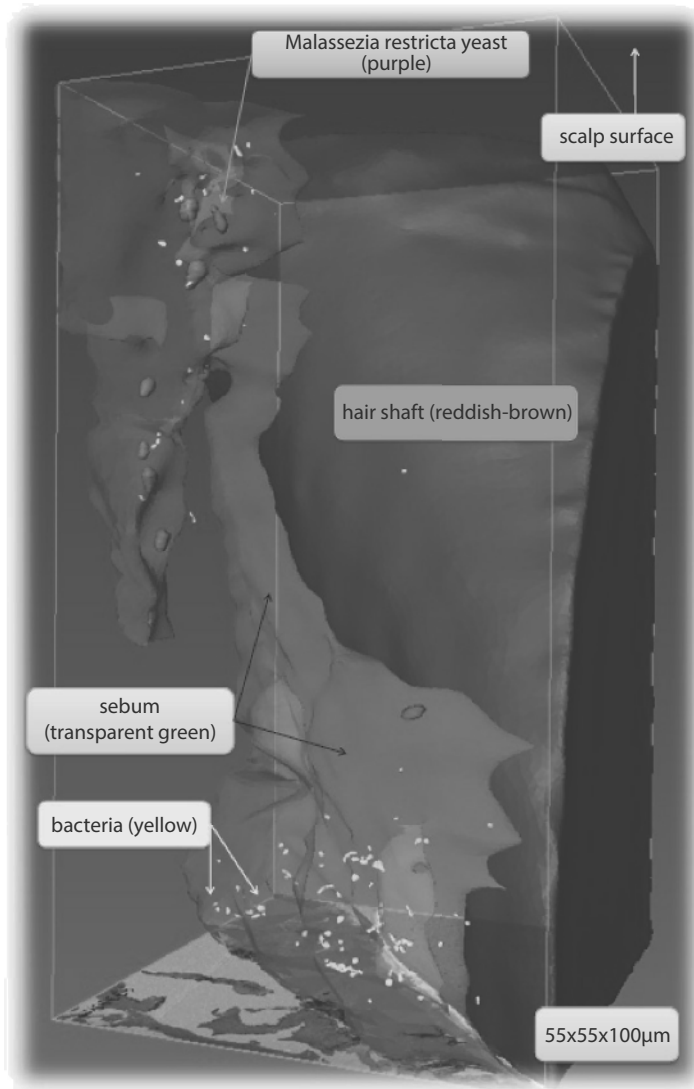


Figure 1.1 Use of an SEM image stack to visualise localisation of bacteria and yeast in a hair follicle. Reprinted with permission © Unilever.

However, despite the undoubted contribution of this work, it suffered from the limitations of laboratory culture techniques which restricted the organisms that could be detected and quantified to those that could be reproducibly cultured under laboratory conditions, and failed to capture the true diversity of the skin microbiome [18, 19].

1.1.2 Next Generation Sequencing

The advent of next generation sequencing techniques and advances in bioinformatics have transformed our understanding of the skin microbiome by tackling the reliance of the researcher on the agar plate as their sole tool in elucidating the composition of the skin's microbial ecosystem.

Consequently, rather than simply culturing and examining a few microbial species at a time, it is now possible to examine the entire skin microbiome in a single experiment and the advent of affordable, assessable sequencing has led to a rapid expansion in our understanding of the human skin microbiome [18, 20–23].

The NIH funded Human Microbiome Project (2007–2014) and subsequent Integrative Human Microbiome Project (2014–2016) collected key-stone information on the taxonomic composition of the vaginal, oral, skin and gut microbiomes and subsequently, through the iHMP, insights on host-microbiome interactions [24–27].

Although the work of the HMP played an essential contribution to kick-starting large-scale cohort studies of the human microbiome, the job is far from done. Significant work is needed to expand the clinical space (the iHMP focused on preterm birth, inflammatory bowel disease and type 2 diabetes) and our understanding of the normal cross-sectional and longitudinal variance of the health-associated microbiome.

Whilst the HMP focused primarily on taxonomic characterization, it is likely that future investigations will focus more on functional characterization through the application of metagenomic and combined microbiome/metabolome analysis. This trend is already apparent in gut research where gut microbiome studies, such as MetaHIT in Europe, ElderMet in Ireland, the Canadian Microbiome Initiative and Japanese Human Metagenome consortia, all focused on elucidating function [28].

Application of such functional characterization techniques to the skin microbiome is already happening [29, 30] and their use in large-scale cohort studies focused on the skin microbiome and the derivation of this data into a holistic, ecological perspective on host/microbiome looks likely to represent the next new frontier for skin microbiome research [31].

1.2 The Skin Microbiome in Health

1.2.1 Composition

As an ecological substrate, human skin varies enormously across different locations over the body. Sebum-rich sites are found on the face, chest, back and groin. Hair density similarly varies with higher densities on the scalp, underarm and genital areas. Consequently, it should be no surprise that the composition of the human microbiome similarly varies and that body site, by virtue of these ecological differences, plays a key role. This gives rise to the notion that the skin microbiome may be more properly considered as a composite of the interrelated but distinct microbiomes of the scalp, leg, axilla, face, etc. [22, 23, 32, 33].

Comparing across certain body sites, we see that a niche of specific microbial ecology characteristics may be observed that is driven by the physiological conditions present at each site (Figure 1.2). The skin microbiome of all body sites is expected to contain representatives from the genera *Cutibacterium*, *Staphylococcus* and *Corynebacterium* and when mean relative abundancies are summed together these three genera may typically comprise between 45 and 80% of the overall skin microbiome and thus

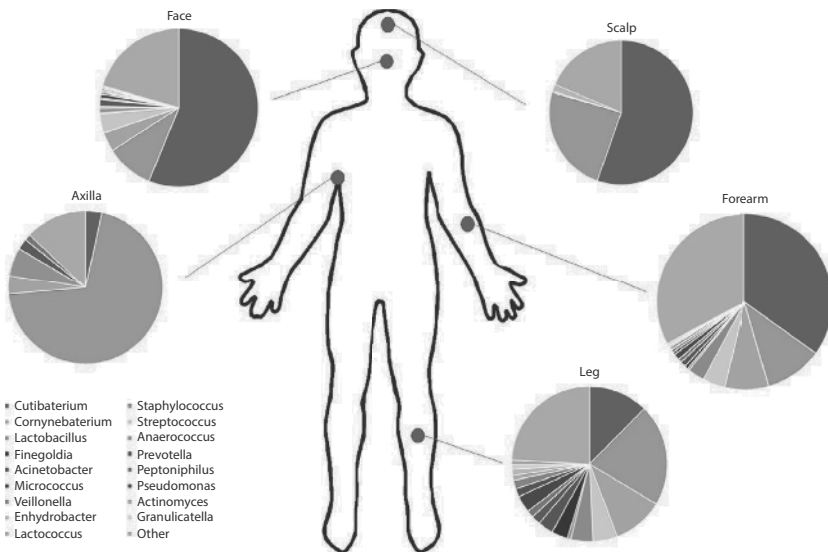


Figure 1.2 Genus level bacterial composition of different body sites as characterised by 16S rRNA gene sequencing (or metataxonomics).

may be considered as being good candidates for any consideration of what a “core” skin microbiome might look like.

However, even within these “big three” genera, important differences in microbiome profile between body sites are apparent. In the axilla moist, occluded sites staphylococci dominate, comprising over 70% of the microbiome in terms of mean relative abundance whilst lipophilic cutibacteria comprise less than 4% of the total bacterial microbiome.

In contrast, in sebaceous body sites the situation is, if not quite reversed, then certainly more favorable to cutibacteria. On both the face and scalp, cutibacteria are the dominant genera, comprising over 50% of the microbiome in terms of mean relative abundance and staphylococci less than 25%.

These changes serve to illustrate the importance of the local microenvironment, particularly the importance of skin sebaceous lipids, skin pH and occlusion/hydration in creating the conditions which define the “normal” or “steady state” microbiome balance which is characteristic of a particular cutaneous niche.

Such changes impact not only the balance of cutibacteria and staphylococci but also the overall diversity of these niche specific microbiomes with sebaceous and occluded sites being more likely to possess an individual genera comprising more than 50% of the microbiome in terms of relative abundance, whilst drier sites appear to be more refractive to any individual genera achieving dominance, which is likely to contribute to the greater microbial diversity observed in these sites.

A similar trend is apparent when the skin microbiome is examined at the species level with generally more species represented (principle genera being *Cutibacterium*, *Staphylococcus* and *Corynebacterium*) in the microbiome of body skin relative to sebaceous or occluded sites (Table 1.1A, B).

Examining the species composition in more detail, we also observe that just as *Cutibacterium*, *Staphylococcus* and *Corynebacterium* are compositionally dominant at the genus level that within these genera the microbiome profile is also skewed to one compositionally dominated by a relatively small number of species with *Cutibacterium acnes* the dominant cutibacteria, *Staphylococcus epidermidis* and *Staphylococcus hominis* the dominant staphylococci.

Although the majority of studies focus on the bacterial microbiome due to the relative maturity of methods, databases and bioinformatic analysis of bacterial 16S rRNA gene sequence data, the fungal microbiome (also referred to as the mycobiome) should also be considered [34].

In comparing the bacterial and fungal skin communities a striking observation is one of diversity. In the case of bacteria it is frequently observed that, for a given body site niche, that one, two or three genera are numerically

Table 1.1 Species level bacterial microbiome of healthy skin (A) Leg, (B), Axilla.
(A)

Cutibacterium		Staphylococcus		Corynebacterium	
Cutibacterium acnes	91%	Staphylococcus hominis	32%	Corynebacterium striatum	19%
Cutibacterium acidifaciens	5%	Staphylococcus epidermidis	28%	Corynebacterium pseudogenitalium/ tuberculostrictum	18%
Cutibacterium granulosum	2%	Staphylococcus haemolyticus	9%	Corynebacterium kroppenstedtii	8%
Cutibacterium propionicum	1%	Staphylococcus capitis/caprae/ epidermidis	8%	Corynebacterium urealyticum	6%
Cutibacterium avidum	1%	Staphylococcus equorum	6%	Corynebacterium lipophiloflavum	5%
Other Cutibacteria	1%	Other Staphylococci	17%	Other Corynebacteria	43%

(B)

Cutibacterium		Staphylococcus		Corynebacterium	
<i>Cutibacterium acnes</i>	68%	<i>Staphylococcus epidermidis</i>	78%	<i>Corynebacterium_sp</i>	100%
<i>Cutibacterium acidifaciens</i>	32%	<i>Staphylococcus hominis</i>	13%		
		<i>Staphylococcus lugdunensis</i>	4%		
		<i>Staphylococcus haemolyticus</i>	1%		
		<i>Staphylococcus sp</i>	3%		

dominant and that there is a “long tail” of genera that are less abundant but still frequently observed at greater than 1% when measured in terms of mean relative abundance. In contrast, the fungal skin mycobiome is, almost regardless of body site niche, overwhelmingly dominated by a single genus, *Malassezia*, a basidiomycete yeast. Whilst other fungi may be detected, including *Candida*, *Trichophyton*, *Rhodotorula* and *Epicoccum*, they are, in healthy skin, a very small part of the overall skin fungal mycobiome [35].

The most comprehensive study of the human skin mycobiome available at the time of writing, conducted by the U.S. National Institute of Health [36], suggests that nearly all cutaneous sites are overwhelmingly numerically dominated by *Malassezia* yeasts, with this species often accounting for more than 90% of the skin fungal mycobiome as measured by mean relative abundance [36], confirming the numeric dominance of *Malassezia* observed by earlier work conducted using qPCR [37]. Indeed, the only body sites where *Malassezia* was not overwhelmingly numerically dominant were the feet (planter heel, toenail and toe-web space), an exception that may be attributed to the dependency of nearly all species of *Malassezia* on an exogenous supply of metabolizable fatty acids [38].

The genus *Malassezia* currently comprises over 14 cultured species [39, 40], of which *Malassezia restricta*, *Malassezia globosa*, *Malassezia slooffiae* and *Malassezia sympodialis* are the predominant species found on human skin [36]. The ratios of these organisms can vary between body sites with *M. slooffiae* and *M. sympodialis* being more abundant on less sebaceous sites [36, 41], potentially due to their less stringent requirements for exogenous lipids [42]. In contrast, *M. restricta* and to a lesser extent *M. globosa* are specialists which thrive in body site niches, such as the scalp and face, rich in sebum and capable of supporting their lipophilic metabolism [40, 43, 44].

To date, the majority of microbiome research has focused on the bacterial community and to a lesser extent, the fungal community. Completing our understanding of microbiome composition is likely to require characterizations of the viral community or virome [45–47]), as well as that of higher organisms such as *Demodex folliculorum*. However, although the microbiome jigsaw is not complete without these elements, we should caution against the belief that without them we are unable to draw useful conclusions, as to do so would be an unnecessary impedance to scientific research.

1.2.2 Diversity

The diversity of any microbiome is typically measured through a combination of alpha diversity (diversity within communities) or beta diversity

(diversity between communities) metrics or by analysis of the number of observed operational taxonomic units (OTUs) [20], and the same measures can be readily applied to analysis of skin microbiome data.

Examinations of skin microbiome diversity in non-compromised skin indicate reproducible differences in microbiome diversity between body sites and also show how local changes in skin ecology, such as changes in oiliness, or barrier integrity can help to explain person to person differences in the same body site.

In their analyses of body site differences, Shibagaki *et al.* [48] demonstrated that the cheek, forehead and scalp typically display lower microbiome diversity than the forearm, and in our own analysis of body site differences (Figure 1.3) our team observed that, in healthy skin, the forearm and leg are typically more diverse than the forehead.

Such observations are consistent with the interpretation proposed in the previous section that sebaceous skin sites are, under normal conditions, less diverse habitats than non-sebaceous sites [49].

This analysis can be taken one step further through the work of Mukherjee *et al.* [50] by looking at diversity differences within an individual body site and comparing that data to person-to-person differences in skin oiliness.

In this study of 30 healthy female subjects, we see not only that a significant positive correlation is observed between *Cutibacterium* sp. and sebum

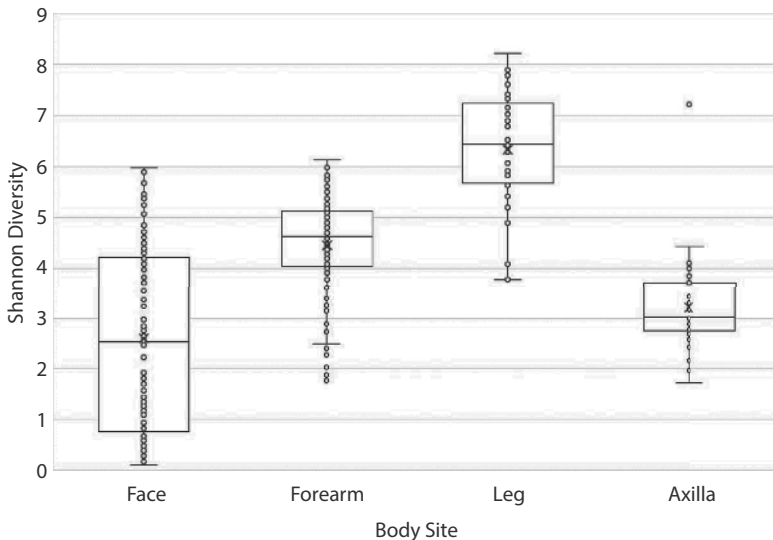


Figure 1.3 Body site differences in microbiome diversity.

levels (Sebumeter, Courage + Khazaka electronic GmbH), but also a step-wise reduction in microbiome diversity as facial sebum levels increase (Figure 1.4).

These insights taken together nicely show not only how the local micro-environmental conditions of different body-site niches act as key drivers in determining normal microbiome diversity but also cautions against the assumption that higher microbiome diversity is inherently “better” than lower microbiome diversity.

A more considered interpretation would be to base conclusions first on an assessment of the normal level of microbiome diversity associated with an individual body site niche under the healthy conditions of non-compromised skin, and then to determine if changes in the diversity of that microbiome, either an increase or decrease in diversity is associated with changes in the underlying skin condition. A deeper level of understanding may be to probe the functional behavior of the various species, meaning judging “health” and “disease” by biota functionality and ideally correlation to clinical manifestation and not by its identity, diversity or distribution.

When such analyses are undertaken, sensible conclusions can be drawn, be they the increase in body site specific microbiome diversity observed in aged skin [48], the conflicting data on changes in microbiome diversity in atopic skin which may either be unchanged [29] or reduced [51] or the lack of significant changes in diversity when comparing normal skin to mild dry skin [52].

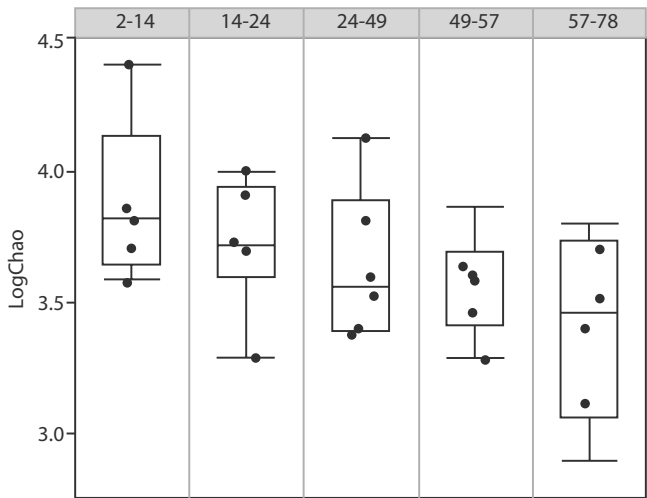


Figure 1.4 Increased skin sebum associates reduced microbiome diversity.

1.2.3 Uniqueness

The uniqueness of each person's individual microbiome is a frequently cited perspective in the scientific and popular press [53–56], and indeed this very uniqueness is being explored for practical application in personalized medicine and forensics [57–61].

However, whilst every person's microbiome may be unique they are not totally dissimilar from one another, indeed much of the core elements of the skin microbiome are common between individuals and populations and the very fact that one can conduct clinical investigations which can characterize the “average” microbiome of a population or which can compare the microbiomes of different body sites [32] supports the hypothesis that, although unique, the differences between the microbiomes of individuals are not so great nor so variable as to confound our attempts at clinical or epidemiological analysis.

Superficially, the notion that an individual's microbiome can simultaneously be unique and sufficiently similar to other people as to permit population-based analyses may appear to be a contradictory one; however, in reality these concepts are able to coexist quite happily.

Perhaps a useful analogy at this point is that of the fingerprint, wherein the unique pattern of whorls, ridges and valleys is, through complex analysis, quite capable of identifying one individual within a population of

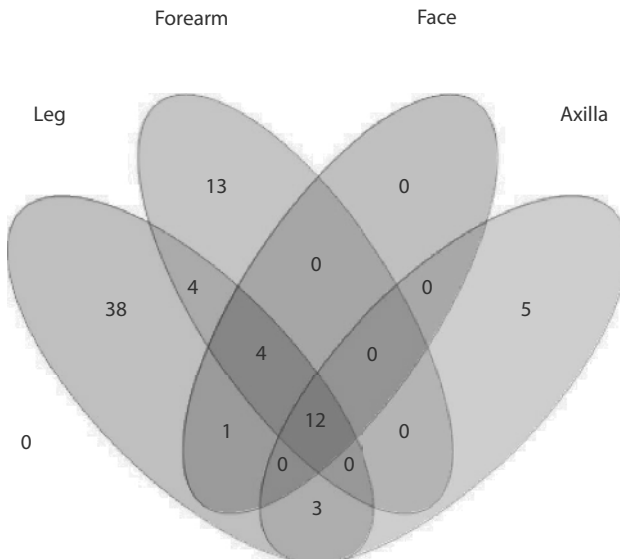


Figure 1.5 Unique and shared genera between body sites.

billions. However, despite their uniqueness in the fine details, fingerprints still remain sufficiently generic for a lay person without any specific training to identify a fingerprint as being “a fingerprint.”

Similarly, in the fine details of the microbiome there are unique, abundant patterns spanning potentially many hundreds or thousands of individual genera or species capable of identifying individuals and potentially highlighting therapeutically important changes in microbiome composition or function. However, by stepping up a length scale one can observe the commonalities between individuals and body sites (Figure 1.5) which are characteristic of the healthy human population.

1.3 Healthy Skin is the Foundation of a Balanced Skin Microbiome

To the skin microbiome the skin is the primary source of nutrients, the primary determinant of microenvironmental conditions such as pH, water availability, temperature and osmolality, the key determinant of physical stability through desquamation and the major source of naturally derived antimicrobial peptides and lipids. Through these processes skin is the curator, shepherding microbiome development and maintaining normal processes of healthy skin development and is the foundation of a balanced skin microbiome.

The skin is composed of several layers, the living tissue of the dermis and epidermis and the outer facing layer of terminally differentiated desquamating keratinocytes called the stratum corneum. Inlaid into this landscape are the many thousands of specialized secretory integuments comprised of eccrine and apocrine glands and terminal and vellus hair follicles [62–64].

The architecture of skin and the associated microbiome are increasingly being viewed not as two separate entities but rather as part of a holistic whole wherein the microbiome forms an outward facing layer atop normal healthy skin, which performs a range of important biochemical and protective functions [65] which will be discussed in more detail later.

1.3.1 Physical Aspects of Skin Impacting the Microbiome

The terminally differentiated keratinocytes comprise the “bricks” of the brick and mortar construction of the stratum corneum, with the mortar comprised of lipids such as ceramides, fatty acids and cholesterol organized in lamellar structures [66, 67].

Although the primary role of the stratum corneum is to prevent water loss from the body and protect the skin by creating a physical barrier to water, the structure is also a key pillar in how healthy skin manages its microbiome, discouraging pathogens and maintaining the presence of commensal organisms [68].

As keratinocytes move outward they undergo a process of terminal differentiation, expressing specialized keratins such as K1 and K10, and late differentiation markers such as involucrin, loricrin and filaggrin, followed by the breakdown of intracellular organelles and conversion of filaggrin into a mixture of filaggrin-derived natural moisturizing factors (NMFs) [69]. Finally, in the last stages of keratinocyte differentiation a finely controlled balance of desquamatory proteases cleaves the protein “rivets” or corneodesmosomes linking stratum corneum cells (squames) together, leading to the controlled shedding of dead skin cells, matching the rate of new cell production and thereby maintaining a constant thickness of stratum corneum [70–72].

This fundamental process, which is so characteristic of normal skin, also has profound implications on the skin microenvironment and thus on the skin microbiome.

The process of desquamation, wherein one layer of skin cells are effectively shed each day means that any adhered microorganisms are similarly shed, creating a biological chemostat selecting for those specialized skin commensal organisms capable of both adhering to skin cells and reproducing at a sufficient rate to maintain a stable community [73]. This process may help to explain why persistent skin-associated biofilms are usually confined to invaginations such as hair follicles [15].

In addition to desquamation, as keratinocytes differentiate free water already localized to the cell interior becomes nearly completely bound by lipids and, importantly, by amino acids including the natural moisturizing factors (NMFs), creating a highly arid environment that is functionally devoid of free water [74].

The exceptionally low water availability of the skin surface combined with high concentrations of stratum corneum and eccrine amino acids (up to 0.3M) [75] combine to create an environment with high osmolarity [73], further increasing the risk of desiccation posed to any resident microorganism.

Potentially as a result of the low availability of free water on the surface of normal healthy skin changes in skin hydration can have a significant effect on the human microbiome [76]. Studies have also demonstrated that skin hydration, as measured by corneometer, impacted the proportions of cutibacteria and staphylococci on the face [50] and aerobic bacteria on hands [77].

Thus, maintaining the physical microenvironment of the stratum corneum of healthy skin creates powerful selective pressures which help to tailor the skin microbiome by permitting those organisms specialized to survive in such conditions the opportunity to flourish whilst discouraging non-commensal opportunists.

1.3.2 Biochemical and Defensive Aspects of Skin Impacting the Microbiome

1.3.2.1 *The Acid Mantle*

Healthy skin maintains a mildly acidic pH through a combination of releasing polycarboxylic acid from filaggrin, the liberation of free fatty acids endogenously from phospholipids and via hydrolysis of sebaceous triglycerides by the commensal microbiome and the release of lactic acid from eccrine glands, all of which contribute to the skin's ability to maintain a remarkably stable acidity [78, 79].

This acidic layer helps to discourage non-commensal species with commensals evolving numerous strategies to facilitate survival, including the use of arginine deiminase pathways to generate ammonia through the conversion of ornithine to arginine [80].

Typically, skin pH values range from 4.0 to 6.0, with normal healthy pH around 5.0. Microbes on the skin are dependent on the slightly acidic pH of the skin. For example, experimental studies show that the commensal *S. epidermidis* grows well under acidic conditions (low pH), but an increase in pH may lead to it being outcompeted by potentially pathogenic species such as *S. aureus* [81].

Changes in skin pH can also impact the balance between commensal species, as has been observed with skin staphylococci and corynebacteria with species of the latter genera being less tolerant of more acid skin pH and tending to be most abundant in body sites like the axilla, which typically exhibits a slightly elevated pH relative to other body sites [82].

1.3.2.2 *Antimicrobial Lipids (AMLs)*

Healthy skin produces a range of antimicrobial free fatty acids and sphingolipids displaying natural antimicrobial activity.

Triglycerides produced in the sebaceous glands [13] are hydrolyzed by lipases secreted by the skin microbiome, principally by *Cutibacterium acnes* [11] releasing free fatty acids which then disperse over the surface of skin (Figure 1.6).

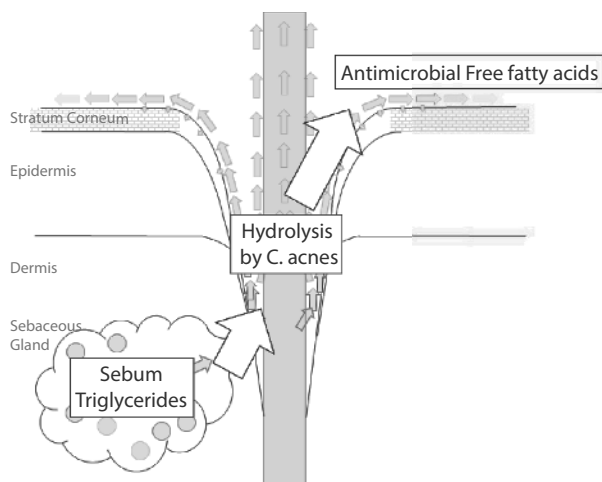


Figure 1.6 The critical role of *Cutibacterium acnes* in liberating antimicrobial free fatty acids from sebum.

In humans, the major free fatty acid liberated in this way is a mono-unsaturated 16:1 fatty acid called sapienic acid (16:1 Δ 6) [83], which has been shown to be antimicrobial against the skin pathogen *S. aureus* *in vitro* [84–87]. Furthermore, levels of this antimicrobial fatty acid are reduced in atopic skin, with levels of sapienic acid *in vivo* negatively correlating with *in-vivo* counts of *S. aureus* [88].

The other major type of antimicrobial fatty acid is sphingosine which is liberated from stratum corneum ceramides by the action of acid and alkaline ceramidases [89]. Like sapienic acid, sphingosine has been shown to have antimicrobial efficacy *in vitro* [87] and to be reduced in atopic skin (150 μ M vs 270 μ M in healthy skin), with this decline being correlated with decreased acid ceramidase activity [89].

Antimicrobial lipids are discussed in more detail in Chapter 11 of this book.

1.3.2.3 Antimicrobial Peptides (AMPs)

Antimicrobial peptides are a family of small endogenously produced compounds present in the stratum corneum and released through sweat and sebaceous secretions which form a protective layer characterized by direct antimicrobial activity [90, 91], which facilitates the formation of epithelial tight junctions [92] and which may be promoted by the presence of commensal microorganisms [80, 93].

Principle AMPs of human skin include cathelicidins such as LL-37 characterized by an N-terminal signal peptide, dermcidins characterized by a cysteine residue disulfide bridge, the S100 protein, the ribonuclease RNase7 and defensins, notably human-beta-defensins (hBD) 1, 2 and 3, as well as lysozyme and iron-binding proteins such as lactoferrin [94].

The constitutive expression of several AMPs, including LL-37 and hBD-1, hBD-2 and hBD-3, coupled to their antimicrobial function supports the existence of a functional role in which healthy skin uses AMPs as a means to manage the normal composition of the skin microbiome by deterring pathogens and commensal overgrowth. The intricate relationship with normal healthy skin, evidenced by the reported synergy between microbiome-derived antimicrobial compounds with human LL-37 [95] is also evidenced by both the role of the endogenous multifunctional skin proteases Kallikrein 5 and Kallikrein 7 in the enzymatic release of active LL-37 from its precursor proform hCAP18 [96] and the observed increase in skin AMPs following the topical application of niacinamide [97].

Antimicrobial peptides are discussed in more detail in Chapter 12 of this book.

1.3.3 Nutritional and Microenvironmental Aspects of Skin Impacting the Microbiome

The scope thus far has been focused on the barrier and defensive properties of healthy skin and how those elements can shape the microbiome. However, healthy skin is also a source of nutrients which support the growth of, or in some cases are critical to the survival of, the normal skin microbiome. Despite the expected importance of understanding the nutritional linkages between skin and its microbiome, little systematic work has been undertaken in the area and the sort of detailed food webs needed for a thorough ecological understanding of the skin microbiome are largely absent from the scientific literature.

1.3.3.1 Amino Acids

The commensal microbiome, including *Micrococcus*, *Staphylococcus*, *Cutibacterium* and *Malassezia* has a requirement for organic nitrogen that can be fulfilled by endogenously produced amino acids [76]. Millimolar concentrations of amino acids are exuded onto skin every day in the form of eccrine sweat (Figure 1.7), providing rich supplies of serine, glycine, alanine and other amino acids [64] and similar amounts provided from

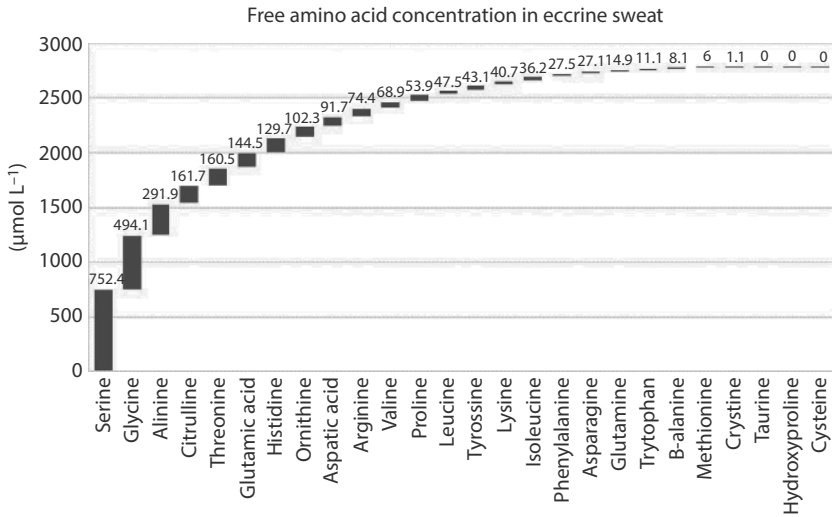


Figure 1.7 Free amino acid composition of eccrine sweat (reproduced from Harker & Harding 2013).

below via the stratum corneum [75], which can provide usable substrates for growth of commensal *Staphylococcus epidermidis* and the skin pathogen *Staphylococcus aureus* [98].

1.3.3.2 Sebaceous Lipids

The levels of lipids in the skin can affect microbiome diversity [48, 50, 99]. Some microbes, such as *Cutibacterium* and *Malassezia*, are particularly dependent on lipids for growth and co-localize with areas of the body where such nutrients are abundant [30, 76]. During puberty, the sebaceous gland becomes activated [100], leading to a shift from a diverse fungal community to one that is dominated by *Malassezia* species [101]. *Cutibacterium acnes* is also notably more abundant in sebaceous regions of skin [76, 102].

1.3.3.3 Organic Acids and Other Materials

Lactate and urea are both abundant on skin, the former from eccrine sweat and the latter as a by-product of lactate/citrulline cycling in the skin surface urea cycle [103] and may support growth of skin bacteria such as *Acinetobacter* [73], which may in part contribute to the above expected presence of urease metabolism pathways in skin bacteria [104].

1.4 A Balanced Skin Microbiome Supports the Normal Functioning of Healthy Skin

Perhaps it should come as no surprise given the millennia over which our skin and skin microbiome have co-evolved, but as our understanding improves it is becoming increasingly apparent that the microbiome of healthy skin is not an inert layer or passenger but is instead an active contributor to the development and maintenance of healthy skin.

This section will explore the role of the microbiome in maintaining healthy skin; from supporting the immune system, to helping maintain skin's normal pH, to the indirect benefits that come from metabolizing various compounds present in the skin.

1.4.1 Pathogen Exclusion

One of the most well-recognized beneficial functions of the commensal skin microbiome is to help prevent colonization of skin by pathogenic microbes [65, 73, 105].

Several different mechanisms contribute to this beneficial effect, in its simplest form this is a question of ecological real estate, whereby the normal skin microbiome, by utilizing resources (nutrients and space) that could otherwise be taken up by pathogens, makes it harder for less desirable microorganisms to gain a foothold on healthy skin [73].

In addition to the sort of passive exclusion described above, the commensal microbiome also plays a direct, active role in dissuading pathogen colonization through the production of bacteriocins and short chain fatty acids (SCFAs) which impede pathogen survival [106, 107]. This is well illustrated by the relationship between *S. epidermidis* and *S. aureus* where competition for nutrients and adhesion receptors [108] can inhibit the formation of, or destroy existing, *S. aureus* biofilms through the production of phenol-soluble modulins [109–111].

Finally, the commensal microbiome may utilize an indirect, skin mediated mechanism to prevent pathogen colonization by amplifying the innate immune response to pathogens [93] or by releasing serine proteases which promote the production of the host endogenous antimicrobial peptides, including human beta defensins 2 and 3, which have been shown to target the skin pathogen *S. aureus* [112, 113].

1.4.2 Contribution to Skin pH

The contribution of cutibacteria and to a lesser extent staphylococci builds on early work demonstrating the role of these organisms in the hydrolytic

generation of free fatty acids [11, 13] and later work on the contribution of free fatty acids to the maintenance of skin pH [79]. *In vivo*, these members of the normal skin microbiome produce lipase enzymes that break down sebaceous triglycerides, releasing fatty acids such as sapienic acid and butyric acid [83, 114]. These fatty acids provide important functions in maintaining pH, exerting direct antimicrobial effects and support the function of the skin barrier by contributing to its acidic pH [79].

1.4.3 Microbial Contribution to Skin Barrier Integrity

In addition to their contribution to skin pH and hydration through the hydrolysis of sebaceous triglycerides (described above) and the deterrence of pathogens through the production of short chain fatty acids (SCFAs) [49, 115], the commensal microbiome has been reported to contribute to skin barrier integrity via a number of different mechanisms.

Human autologous inoculation studies were performed, wherein cultures of the commensal skin microorganisms *S. epidermidis* were recovered from individuals, cultured and applied back to the facial skin of test subjects. These investigations have demonstrated that subjects receiving a topical “top-up” of *S. epidermidis* exhibited a 5–15-fold increase in the number of this organism and also significant improvements in skin condition across a range of skin barrier metrics, including water content, evaporative water loss and lipid content [116].

Separately, investigations into the topical application of *Streptococcus thermophilus* have lent support to the potential role of skin microorganisms in supporting ceramide production via the action of bacterial sphingomyelinase. Through a series of experiments involving application of the organism to *in-vitro* keratinocytes and *in-vivo* healthy and atopic subjects, this work offers an interesting perspective into how the microbiome may play a supportive role in key processes of skin barrier formation [117–119].

An emerging area of research with intriguing potential is the apparent contribution of the commensal skin microbiome to the development of normal skin. Although such work is in its early stages, results such as those of Meisel *et al.* [120] where genes of the epidermal differentiation complex (EDC)—a gene complex enriched in genes encoding for various stages of keratinocyte cornification and which in humans is located on chromosome 1q21—were differentially expressed in a comparison of germ-free and conventionally raised mice, are suggestive of a positive contribution from the commensal microbiome.

In addition, *in-vitro* studies using laboratory grown keratinocytes suggest that skin pathogens actively degrade epidermal tight junctions

(an integral part of the living barrier of skin), whereas commensal organisms did not [121], and that in addition, the expression of antimicrobial peptides (see Subsection 1.3.2.3) was also associated with increased tight junctional development [122, 123].

1.5 Conclusion

This is truly an exciting time to be a microbiologist. The sequencing revolution which gave life to the new science of microbiomics also prompted a renaissance in skin microbiology and the opportunity to revisit past insights equipped with new data and the tools of modern science.

In our team's opinion, one of the most profound changes arising from this rejuvenation of interest in the skin microbiome is the shift away from a monolithic view of skin microorganisms as being either negative actors or passive bystanders and towards a holistic perspective embracing the beneficial interplay between skin and skin microbiome in the maintenance of healthy skin (Figure 1.8).

Despite the work of the HMP increasing the number of academic and commercial research projects focused on the skin microbiome, gaps in our understanding remain.

One of these is the ongoing need to ensure we have skin microbiome data which is properly representative of the diversity of skin and which

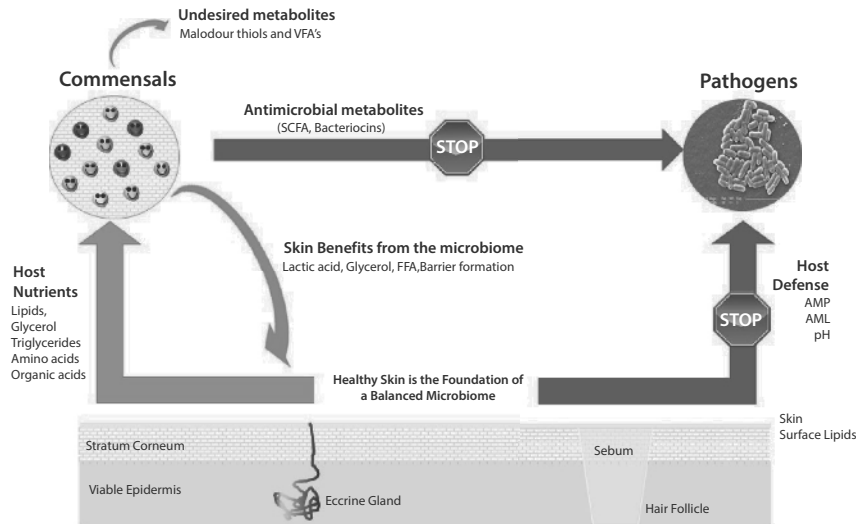


Figure 1.8 Holistic interaction of skin and microbiome.

properly reflects the diversity of human ethnicities, ages, lifestyles and climatic conditions. Without a concerted effort in this area we run the risk of overlooking new associations between skin and microbiome.

Another area is the need to build a more complete understanding of skin microbiome function grounded in a more holistic interpretation of “microbiome ecology” that takes new insights such as those emerging from the combined microbiome/metabolome studies of Pieter Dorrestein [30] to build an ecological perspective on human-microbiome and microbe-microbe interactions within the microbiome.

Acknowledgments

The authors would like to express gratitude to the following individuals for their contributions to this chapter and the previously unpublished scientific studies reported therein: Dr. Barry Murphy and Dr. David Arnold (Unilever R&D Port Sunlight); Dr. Gordon James (Unilever R&D Colworth); Drs. Anindya Dasgupta, Amitabha Majumdar and Rupak Mitra (Unilever R&D Bangalore); Dr. Cheri Chi (Unilever R&D Shanghai); and Dr. Stacy Hawkins (Unilever R&D Trumbull).

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