Skin Microbiome Handbook
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Martin Scrivener (martin@scrivenerpublishing.com)
Phillip Carmical (pcarmical@scrivenerpublishing.com)
This book is dedicated to my one and only ever and forever, my husband and partner, and to my two amazing children. My husband was the one who seeded in me the idea to edit this book. He and my children teach me, every day, the practice of unconditional love and support and, as such, they are my mentors to connect to God.
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Preface

I belong to those scientists who believe in the existence of God and as such I know we are here to connect to Him, love and cherish Him and His creation. Exploring nature through research is merely a way of understanding the Creator. While humans can invent extraordinary creations, these are only a revelation and exploration of His work. It also means that we are extremely limited. Humility is at the core of our work.

Our senses dictate to a great degree the reality we live in. Yet, as educated human beings in the scientific era, we acknowledge the fact that the existence of another entity or power in our life, even if not sensed or seen, can be real. The microbiome is a dimension of our reality that is alive and vibrant but cannot be seen by the naked eye. An entire microcosmic universe of activity affects every aspect of our being, from the planet to our bodies to our spirit and back. With the invention of high-resolution techniques, such as microscopy, we began to learn about these entities. With the immense advancement in genomic research, we are now making progress in exploring their nature and identity.

Our ability to sequence the genome in a faster and more economically savvy fashion has been greatly promoted by the human genome project, which gave rise to this new level of exploration of entities with genomic material that is different from humans, such as the microbiome. In the end, we are all connected.

Of more importance is the profound acknowledgment of the power and influence that the microbiome holds over our health and well-being. These microorganisms can make the difference between life and death, health and disease, depression and mania... The list goes on! This book is written at a time when the research is still shaping our knowledge, and as such, it can be perceived as a milestone at a stage where we know the basic nature of the players but are still at the edge of exploring the interplay in the scene. Bacteria, viruses and fungi communicate. They communicate with one another and they communicate with us at the cellular and sub-cellular levels when on us or inside us. This cross talk is what I believe the next era of
research will focus on. In a sense, the identity of the communicator (bacteria or human cell) is mute when compared to what it conveys and why. In research we call it “functionality.” Take for example a bacterium that contains genetic material of about 2500 protein encoding genes. In theory, it has the potential to generate 2500 proteins that will function as receptors, toxins, enzymes and other biomarkers. These can be recognized by human cells that will respond in accordance to the message carried with the biochemistry produced. The environment is the compass for the bacteria to act in one way or another. This is the epigenetics of the human body as an ecosystem that contains both human cells and microorganisms.

If there is an imminent safety threat, it will create a protection *weapon* in the form of toxins. If it is well nourished and safe, it may facilitate a beneficial immune response that will strengthen our bodies.

From a practical evolutionary point of view, the microbiome that resides in a healthy human body would have an agenda of survival and proliferation, and as such, would strive to protect the body and maintain its health so that symbiosis persists.

The idea of good and bad, protection and nourishment, health and disease, survival and death are all God’s creation embedded in us at a molecular, cellular, and sub-cellular level. In a sense, every part of us, however big or small, is on a journey to explore the higher levels of conciseness. After a decade of studying the skin microbiome, I am convinced that we limit our understanding because we attribute to it aspects of human nature. Humans are the only entity in this world that have been created with an ego. As generations advance, the ego has now grown to monstrous dimensions. Bacteria, on the other hand, does not hold these same aspirations. Rather, it is busy with the very basics of survival. Adopting this understanding may allow us breakthrough revelations.

This book, similar to my other books, is a compilation of knowledge and experience of good colleagues. They are all experts in the field and I am extremely thankful for their hard work and dedication.

It covers various aspects in observational and interventional studies, health and disease conditions, testing techniques, human body response, as well as legal and regulatory outlooks.

I can only hope that you, the reader, will experience the same joy of learning as I did while editing this book.

*Nava Dayan*

June 2020
Part 1

HEALTHY SKIN MICROBIOME
AND ORAL-SKIN INTERACTIONS
1

The Microbiome of Healthy Skin

Samantha Samaras* and Michael Hoptroff†

1Beauty & Personal Care Science and Technology, Unilever, United States
2Beauty & Personal Care Science and Technology, Unilever UK Limited, UK

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

*Corresponding author: Samantha.Samaras@Unilever.com
†Corresponding author: Michael.Hoptroff@Unilever.com

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 microorganisms 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, assessible 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 keystone 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](image)

*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.

<table>
<thead>
<tr>
<th>Cutibacterium</th>
<th>Staphylococcus</th>
<th>Corynebacterium</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cutibacterium acnes</td>
<td>91%</td>
<td>Staphylococcus hominis 32%</td>
</tr>
<tr>
<td>Cutibacterium acidifaciens</td>
<td>5%</td>
<td>Staphylococcus epidermidis 28%</td>
</tr>
<tr>
<td>Cutibacterium granulosum</td>
<td>2%</td>
<td>Staphylococcus haemolyticus 9%</td>
</tr>
<tr>
<td>Cutibacterium propionicum</td>
<td>1%</td>
<td>Staphylococcus capitis/caprae/epidermidis 8%</td>
</tr>
<tr>
<td>Cutibacterium avidum</td>
<td>1%</td>
<td>Staphylococcus equorum 6%</td>
</tr>
<tr>
<td>Other Cutibacteria</td>
<td>1%</td>
<td>Other Staphylococci 17%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Other Corynebacteria 43%</td>
</tr>
<tr>
<td>(B)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cutibacterium acnes</td>
<td>68%</td>
<td>Staphylococcus epidermidis 78%</td>
</tr>
<tr>
<td>Cutibacterium acidifaciens</td>
<td>32%</td>
<td>Staphylococcus hominis 13%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Staphylococcus lugdunensis 4%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Staphylococcus haemolyticus 1%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Staphylococcus sp 3%</td>
</tr>
</tbody>
</table>
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...